Example 2

This example describes the isolation of a potent major phase II enzyme inducer from broccoli.

Fractionation of acetonitrile extracts of SAGA broccoli by preparative reverse-phase HPLC with a water/methanol solvent gradient resulted in recovery of 70-90% of the applied inducer activity in the chromatographic fractions. Surprisingly, the majority (about 65-80% in several chromatographies) of the recovered activity was associated with a single and relatively sharp peak [fractions 18-23; eluted at 64-71% (vol/vol) methanol]. This HPLC procedure was therefore adopted as the first step of the larger-scale isolation of inducer activity.

Lyophilized SAGA broccoli was extracted three times with acetonitrile (35 ml/g) for 6 hr each at 4°C. The pooled extracts were filtered successively through 0.45- and 0.22-μm porosity filters (discarding the insoluble material) and evaporated to dryness under reduced pressure on a rotating evaporator (<40°C). About 1 g of residue from 640 g of fresh broccoli (64 g of lyophilized powder) contained 3.6×10^6 units of inducer activity. The residue was mixed thoroughly with 120 ml of methanol/water (25/75, vol/vol) and the insoluble fraction was discarded. Although not all of the residue obtained from the extraction was soluble in aqueous methanol, the solvent partition procedure resulted in substantial purification without significant loss of inducer activity. Portions of the extract were dried in a vacuum centrifuge and dissolved in small volumes of dimethyl formamide (0.75-1.0 ml per 50 mg of residue), and 50-mg portions were subjected

to HPLC (nine runs). Fractions 18-23 from all runs were pooled, evaporated to dryness, applied in acetonitrile to five preparative silica TLC plates (100 x 200 x 0.25 mm), and developed with acetonitrile, which was run to the top of each plate three times. Four major fluorescence-quenching components were resolved, and nearly all (99%) of the inducer activity migrated at R_f 0.4. The active bands were eluted with acetonitrile, pooled, and fractionated by two runs on a second preparative reverse-phase HPLC in a water/acetonitrile gradient (20%-71%). Ultraviolet absorption and inducer activity were eluted in a sharp coincident peak (at 66% acetonitrile) that contained all of the activity applied to the column. Evaporation (<40°C) of the active fractions gave 8.9 mg of a slightly yellow liquid, which contained 558,000 inducer units (overall yield 15%) and migrated as a single band on TLC.

Example 3

This example describes the identification of the inducer isolated from broccoli, as described in Example 2.

The identify of the inducer was established by spectroscopic methods and confirmed by chemical synthesis. It is (-)-1-isothiocyanato-(4R)-(methylsulfinyl) butane, known as sulforaphane or sulphoraphane (CAS 4478-93-7). See Figure 1.

Sulforaphane has been synthesized (Schmid, et al., *Helv. Chim. Acta* *31*:1497-1505 (1948)) and isolated from leaves of hoary cress (Procházka, *Collect. Czech. Chem. Commun.* *24*:2429-2430 (1959)) and from other plants (Kjær, et al.,

Acta Chem. Scand. *12*:833-838 (1958)), and the absolute configuration has been assigned (Mislow, et al., *J. Am. Chem. Soc.* *87*:665-666 (1965)). The closely related olefin sulforaphene [4-isothiocyanato-(1R)-(methyl-sulfinyl)-1-(E)butene (CAS 2404-46-8)] has been isolated from radish seeds and other plants (Schmid, et al., *Helv. Chim. Acta* *31*:1017-1028 (1948); Hansen, et al., *Acta Chem. Scand. Ser. B* *28*:418-424 (1974)) and has also been synthesized (Cheung, et al., *J. Chem. Soc. Chem. Commun.*, 100-102 (1965); Balenović, et al., *Tetrahedron* *22*:2139-2143 (1966)).

The following evidence establishes that (R)-sulforaphane is the inducer isolated from broccoli, UV spectrum (H_2O): λ_{max} 238 nm, ϵ_{238} 910 $M^{-1}cm^{-1}$; addition of NaOH (0.1 M) blue-shifted (λ_{max} 226 nm) and intensified (ϵ_{226} 15,300 $M^{-1}cm^{-1}$) this absorption band, consistent with the behavior of isothiocyanates (Svátek, et al., *Acta. Chem. Scand.* *13*:442-455 (1959)). IR (Fourier transform, neat): strong absorptions at 2179 and 2108 cm^{-1} and also at 1350 cm^{-1}, characteristic of isothiocyanates (Kjær, et al., *Acta Chem. Scand.* *9*:1311-1316 (1955)). ^1H NMR (400 MHz, C^2HCl_3): δ 3.60 (t, 2H, J = 6.1 Hz,—CH_2—NCS), 2.80-2.66 (m, 2H, —CH_2—SO—), 2.60 (s, 3H, CH_3—SO—), and 1.99-1.86 ppm (m, 4H, —CH_2CH_2—). ^{13}C NMR (400 MHz, C^2HCl_3): δ 53.5, 44.6, 38.7, 29.0, and 20.1 ppm. Mass spectrometry (fast atom bombardment; thioglycerol matrix) gave prominent peaks at 178 $(M + H)^+$ and 355 $(M_2 + H)^+$. Electron impact mass spectrometry gave a small molecular ion (M^+) at 177, and chemical ionization mass spectrometry gave a small molecular ion $(M + H)^+$ at 178 and

prominent fragment ions with masses of 160, 114, and 72, consistent with the fragmentation pattern shown in Figure 2. Precise masses of molecular and fragment ions obtained by electron impact mass spectrometry were 177.0286 (calculated for $C_6H_{11}NOS_2$, 177.0283), 160.0257 (calculated for $C_6H_{10}NS_2$, 160.0255), and 71.9909 (calculated for $C_2H_2NS_1$, 71.9908). In addition, for the mass 160 fragment, the peaks at 161 (M + 1) and 162 (M + 2) were 8.43% (calculated, 8.44%) and 9.45% (calculated, 10.2%), respectively, of the parent ion. Similarly, for the mass 72 fragment, the peaks at 73 (M + 1) and 74 (M + 2) were 3.42% (calculated, 3.32%) and 5.23% (calculated, 4.44%), respectively, of the parent ion. Hence the isotope compositions corrected for the natural isotope abundance (of ^{13}C, ^{15}N, ^{33}S, and ^{34}S) were consistent with the relative intensities of the M + 1 and M + 2 ions of both fragments. The optical rotation of the isolated material was $[\alpha]^{23}_D$-63.6° (c - 0.5, CH_2Cl_2), thus establishing that the product is largely, if not exclusively, the (—)-(R) enantiomer $[\alpha]_D$ -79°, -73.2°, --66°; refs. 26, 30, and 38, respectively). The spectroscopic properties of synthetic (R,S)-sulforaphane were identical to those of the isolated product.

Example 4

This example describes the synthesis of sulforaphane (CAS 4478-93-7) and its closely related analogs, ibervin, erucin, berteroin, iberin, alyssin, cheirolin, erysolin, and 1-isothiocyanato-5-methylsulfonyl-pentane.

(R,S)-Sulforaphane (CAS 4478-93-7) was prepared according to Schmid and Karrer (Schmid, et al., *Helv. Chim. Acta 31*:1497-1505 (1948)) except that

gaseous thiomethanol was replaced by sodium thiomethoxide. The sulfide analogues, $CH_3—S—(CH_2)_n—NCS$, where n is 4 [erucin (CAS 4430-36-8)] or 5 [berteroin (CAS 4430-42-6)] were prepared as described (Kjær, et al., *Acta Chem. Scand. 9*:1311-1316 (1955)), and the three-carbon analogue [iberverin (CAS 505-79-3)] was prepared from phthalimidopropyl bromide (Schmid, et al., *Helv. Chim. Acta 31*:1497-1505 (1948)). IR spectra of all three sulfide analogues showed strong absorptions near 2150 cm^{-1}, characteristic of isothiocyanates. ^1H NMR spectra of these compounds show sharp singlets at δ 2.10 ppm ($CH_3—S$ group). The sulfoxide analogues where n is 3 [iberin (CAS 505-44-2)] or 5 [alyssin (CAS 646-23-1)] were prepared by the same method as sulforaphane. IR spectra of these compounds showed strong absorptions near 2100 cm^{-1}, assigned to the —NCS group. ^1H NMR spectra also showed sharp singlets around δ 2.5 ppm, consistent with the presence of the $CH_3—SO$ group. The sulfone analogues, $CH_3—SO_2—(CH_2)_n—NCS$, where n is 3 [cheirolin (CAS 505-34-0)], 4[erysolin (CAS 504-84-7)], or 5 (unreported) were prepared by known methods (Schneider, et al., *Liebigs Ann. Chem. 392*:1-15 (1912)). ^1H NMR ($\delta \approx$ 2.9 ppm, for $CH_3—SO—_2—$) and IR spectra of these compounds were consistent with the structures. Every analogue within this example except 1-isothiocyanato-5-methylsulfonyl-pentane [$CH_3—SO_2—(CH_2)_5—NCS$] has been isolated from plants (Kjær, *Fortschr. Chem. Org. Naturst. 18*:122-176 (1960)).

Example 5

This example describes the inducer activity of the closely-related analogs of sulforaphane whose synthesis is described in the preceding Example.

Each of the analogs of sulforaphane was tested for the ability to induce QR in murine hepatoma cells by the assay described in Example 1. The following structure-function relationships were observed.

The chirality of the sulfoxide does not affect inducer potency, since isolated (R)-sulforaphane and synthetic (R,S)-sulforaphane gave closely similar CD values of 0.2-0.4 μM. Sulforaphane is therefore the most potent monofunctional inducer that has been identified (Talalay (1989) *Adv. Enzyme Regul. 28*:237-250; Talalay et al., (1988) *Proc. Natl. Acad. Sci. USA 85*:8261-8265.[3] Both (R)- and (R,S)-sulforaphane were relatively noncytotoxic: the concentrations required to depress cell growth to one-half were 18 μM.

Sulforaphane and the corresponding sulfone (erysolin) were equipotent as inducers of QR, whereas the corresponding sulfide (erucin) was about one-third as active (Table 1). Oxidation of the side-chain sulfide to sulfoxide or sulfone enhanced inducer potency, and compounds with 4 or 5 methylene groups in the bridge linking CH_3S— and —N=C=S were more potent than those with 3 methylene groups.

[3]Benzylisothiocyanate has a reported CD value of 1.8 μM; phenethylisothiocyanate has a CD of 2.0 μM; ethylisothiocyanate has a CD of 30 μM; propylisothiocyanate has a CD of 14 μM; cyclohexylisothiocyanate has a CD of 14 μM.

Table 1. Potency of induction of QR in Hepa 1c1c7 cells by sulforaphane and analogues

Compound	CD value, μM		
	n = 3	n = 4	n = 5
$CH_3-S-(CH_2)_n-N=C=S$	3.5 (Iberverin)	2.3 (Erucin)	1.7 (Berteroin)
$CH_3-S(=O)-(CH_2)_n-N=C=S$	2.4 (Iberin)	0.4–0.8 (Sulforaphane)	0.95 (Alyssin)
$CH_3-S(=O)_2-(CH_2)_n-N=C=S$	1.3 (Cheirolin)	0.82 (Erysolin)	0.98

Mutants of Hepa 1c1c7 cells defective in the Ah (aryl hydrocarbon) receptor or expression of cytochrome P-450IA1 can distinguish monofunctional inducers (which induce phase II enzymes selectively) from bifunctional inducers (which elevate both phase I and II enzymes) (De Long, et al., *Carcinogenesis* 8:1549-1553 (1987); Prochaska, et al., *Cancer Res* 48:4776-4782 (1988)). When sulforaphane was tested with the BP^c1 mutant (Miller, et al., *J. Biol. Chem.* 258:3523-3527 (1983)) (defective in transport of the liganded Ah receptor to the nucleus), and the c1 mutant (Hankinson et al., (1985) *J. Biol. Chem.* 260:1790-1795) (which synthesizes inactive cytochrome P-450IA1), induction of QR was normal (data not shown). Sulforaphane is, therefore, like benzyl isothiocyanate, a monofunctional inducer (Prochaska, et al., *Cancer Res* 48:4776-4782 (1988)) and is unlikely to elevate activities of cytochromes P-450 that could activate carcinogens.

Example 6

This example demonstrates that the anti-cancer agents of the present invention are active in whole animals as inducers of phase II xenobiotic metabolism enzymes.

When synthetic (R,S)-sulforaphane, erysolin, and erucin were administered to female CD-1 mice by gavage (De Long, et al., *Cancer Res.* *45*:546-551 (1985)), induction of QR and glutathione transferase activities was observed in the cytosols of several organs (Table 2). Sulforaphane and erucin (in daily doses of 15 μmol for 5 days) raised both enzyme activities 1.6- to 3.1-fold in liver, fore-stomach, glandular stomach, and mucosa of proximal small intestine, and to a lesser degree in lung. The sulfone (erysolin) was more toxic, but even 5-μmol daily doses for 5 days elevated the specific activities of these enzymes in some tissues examined. We therefore conclude that sulforaphane and its analogues not only induce QR in Hepa 1c1c7 murine hepatoma cells but also induce both QR and glutathione transferase activities in a number of murine organs.

Table 2. Induction of QR and glutathione S-transferase (GST) in mouse tissues by sulforaphane and analogues

Inducer	Dose, μmol per mouse per day	Enzyme	Ratio of specific activities (treated/control): Liver	Forestomach	Glandular stomach	Proximal small intestin	Lung
CH_3—S—$(CH_2)_4$—NCS Erucin	15	QR	2.19 ± 0.06	1.64 ± 0.18*	1.72 ± 0.11	3.10 ± 0.20	1.66 ± 0.1[illegible]
		GST	1.86 ± 0.08	2.51 ± 0.11	2.07 ± 0.08	3.00 ± 0.21	1.41 ± 0.11
CH_3—S(=O)—$(CH_2)_4$—NCS Sulforaphane	15	QR	2.45 ± 0.07	1.70 ± 0.18*	2.35 ± 0.06	2.34 ± 0.19	1.37 ± 0.1[illegible]
		GST	1.86 ± 0.08	1.98 ± 0.08	2.97 ± 0.08	2.13 ± 0.20	1.17 ± 0.09
CH_3—S(=O)(=O)—$(CH_2)_4$—NCS Erysolin	5	QR	1.62 ± 0.09	1.05 ± 0.21†	1.57 ± 0.08†	1.22 ± 0.20†	1.00 ± 0.11
		GST	1.08 ± 0.11†	1.45 ± 0.15†	1.94 ± 0.10†	0.87 ± 0.20†	1.09 ± 0.1[illegible]

The compounds were administered to 6-week-old female CD-1 mice (4 or 5 mice per group) by gavage in indicated single daily doses in 0.[illegible] ml of Emulphor EL 620P (GAF, Linden, NJ) for 5 days. Cytosols were prepared from the tissues 24 hr after the last treatment and assayed fo[illegible] enzyme activities (glutathione S-transferase was measured with 1-chloro-2,4-dinitrobenzene). The specific activities (nmol·min^{-1}·mg^{-1} ± SEM) of organs of vehicle-treated control mice were as follows. Liver: QR, 47 ± 0.70; GST, 1014 ± 69. Forestomach: QR, 1038 ± 155; GST, 118[illegible] ± 74. Glandular stomach: QR, 3274 ± 85; GST, 1092 ± 81. Small intestine: QR, 664 ± 119; GST, 1372 ± 266. Lung: QR, 54 ± 5.8; GST, 43[illegible] ± 34. Data are presented as mean ± SEM. All ratios were significantly different from 1.0 with $P < 0.01$, except for *, $P < 0.05$, and †, $P >$ 0.05.

Example 7

This example describes the synthesis of *exo*-2-acetyl-6-isothiocyanatonorbornane (GHP 1066 and 1067).

From 1a (mixture)

To a 100 ml 3-neck round bottomed flask equipped with a magnetic stirring bar, dropping funnel and reflex condenser were placed 2.0 g (14.7 mmol) of 2-acetyl-5-norbornene (Aldrich Chemical Co.) and 10 ml of benzene. To this solution was added at room temperature (RT) a mixture of 2.1 g of conc. sulfuric acid and 1.0 ml of water slowly using a dropping funnel. After 4 days at RT, the reaction mixture was filtered through a sintered glass funnel. The filtered white solid was washed with 50 ml of ether. The combined organic solution was then washed with water and brine successively, dried over $MgSO_4$, and concentrated in vacuo to afford a tan oil. Subsequent purification via flash silica-gel column chromatography (20/80, ether/hexane) afforded 1.73 g of product (60% yield, colorless oil) as a mixture of 4 stereoisomers based on ^1H NMR analysis (2a: 2b: 2c: 2d 36:39; 8:17). Purification by HPLC (silica-semi prep, 97/3 hexane/EtOAc, 10 ml/min) gave 2a (GHP 1066) in 22% overall yield.

From 1b (exo-only)

The same mixture as the above (1b:0.665 g) was stirred for 40 hr at 50°C. After the same work-up and column chromatography, 0.970 g of product (70% yield) was obtained as a mixture of 4 stereoisomers (2a: 2b: 2c: 2d 32:47; 1:20).

Characterization of 2a (GHP 1006)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (dd, J = 7.6, J = 2.8 Hz, 1H), 2.71(bs, 1H), 2.43(dd, J = 4.5 Hz, J = 3.6 Hz, 1H), 2.31(dd, J = 8.4 Hz, J = 6.0 Hz, 1H), 2.17(s, 3H), 1.83-1.67(m, 2H), 1.58-1.54 (m, 2H), 1.38-1.30(m, 2H); ^{13}C NMR ($CDCl_3$) δ 58.4, 50.9, 46.6, 40.0, 35.4, 33.6, 31.5, 28.8 (CO and NCS were not detected); FT-IR ($CHCl_3$, cm^{-1}) 2955, 2132, 2085, 1708, 1343; HRMS cald. for $C_{10}H_{13}NOS$ 195.0719, found 195.0719.

Characterization of 2b (GHP 1067) ^{1}H NMR (400 Mhz, $CDCl_3$) δ3.61 (dd, J = 7.2, J = 2.8 Hz, 1H), 2.58 (d, J = 4.4 Hz, 1H), 2.53 (d, J = 4.5 Hz, 1H), 2.35 (dd, J = 8.5 Hz, J = 3.4 Hz, 1H), 2.16 (s, 3H), 2.0 (dt, J = 13.1 Hz, 5.0, 1H), 1.91-1.86 (m, 1H), 1.79-1.75 (m, 1H), 1.55-1.52 (m, 1H), 1.37-1.33 (m, 1H), 1.26-1.22 (m, 1H); FT-IR ($CHCl_3$) 2978 cm^{-1}, 2179, 2146, 2085, 1708, 1449, 1343; Anal. cald. for $C_{10}H_{13}NOS$: C, 61.50; H, 6.71; N, 7.17; S, 16.42 found C, 61.64; H, 6.72; N, 7.12; S, 16.53.

Example 8

This example describes the synthesis of 1-isothiocyanato-5-methylsulfonylpentane (GHP 1003).

Preparation of 2

2 was prepared according to the literature procedure, and the spectral data match the literature values. Kjaer et al. *R. Acta. Chem. Scan.* 1955, 1311.

Preparation of 3

3 was prepared according to the literature procedure, and the spectral data match the literature values. Kjaer et al., *supra*.

Preparation of 4

2 was prepared according to the literature procedure, and the spectral data match the literature values.

Preparation of 5 (GHP 1003)

To a flask charged with 50 mg (0.3 mmol) of 4 and 0.8 ml of H_2O were added a solution of 0.03 ml of $CSCl_2$ in 0.3 ml of $CHCl_3$ and 0.5 ml of 5% NaOH at RT. After 30 min, the reaction mixture was extracted with 20 ml of CH_2Cl_2 (10ml x 2). The combined organic solution was dried over $MgSO_4$, concentrated in vacuo, and purified by preparative TLC (6/4 EtOAc/hexane) to afford 16 mg (0.08 mmol) of 5 (GHP 1003, 26% from 3) as white solids. ^1H NMR (400 MHz, $CDCl_3$) δ 3.50 (t, J = 6.4 Hz, 2H), 2.98 (t, J = 4.1 Hz, 2H), 2.86 (s, 3H), 1.87-1.82 (m, 2H), 1.74-1.66 (m, 2H), 1.58-1.52 (m, 2H); FT-IR 3025 cm^{-1}, 2931, 2191, 2097, 1449, 1314, 1132.

Example 9

This example describes the synthesis of *exo*-2-isothiocyanato-6-methylsulfonylnorbornane (GHP 1068).

Preparation of 2a and 2b

The same procedure as described for GHP 1063 was used except that the reaction mixture was stirred for 6 days at 65°C. After work-up, 2a (17% yield)

were isolated by flash column chromatography (silica gel, 100% ether → 100% EtOAc). 2a (GHP 1064) was recrystallized from CH_2Cl_2/ether/hexane to afford ivy-leaf shaped crystals (mp; 142-143°C) in 12% yield. 2b (GHP 1068) was recrystallized from ether to afford small needles (mp; 82-82.5°C) in 4% yield.

Characterization of 2a

^1H NMR (400 MHz, $CDCl_3$) δ 3.66 (t, J=6.8 Hz, 1H), 2.90 (bs 1H), 2.86 (s, 3H), 2.80 (dd, J = 8.0 Hz, 2.8, 1H), 2.12 (td, J = 14.0 Hz, 5.2, 1H), 2.03 (dt, J = 12.0 Hz, 2.2 1H), 1.88-1.62 (m, 5H); FT-IR ($CHCl_3$) 3025 cm^{-1}, 2120, 2073, 1320; Anal. cald. for $C_9H_{13}NO_2S_2$:C, 46.73; H, 5.66; N, 6.06; S;,27.72. found C, 46.74; H, 5.67; N, 6.11; S,27.64.

Characterization of 2b

^1H NMR (400 MHz, $CDCl_3$) δ 3.65 (dd, J = 6.8 Hz, 2.8, 1H), 2.98 (bs, 1H), 2.87 (s, 3H), 2.76 (dd, J = 6.8, 1.2, 1H), 2.58 (bs, 1H), 2.06-1.61 (m, 6H); FT-IR ($CHCl_3$) 3025 cm^{-1}, 2978, 2191, 2120, 2085, 1349, 1308, 1138.

Example 10

This example describes the synthesis of *cis*-1-isothiocyanate-4-methylsulfonylcyclohexane (GHP 1073).

Preparation of 2

In an autoclave were placed 11.38 g (94.0 mmol. 10 eq.) of butadiene sulfone, 1.00 g (9.4 mmol) of 1, 0.2 g of hydroquinone as a polymerization inhibitor and 20 ml of absolute EtOH. Upon stirring for 15 min., the reaction mixture was sealed and heated at 110°C. After 60 hr., the reaction mixture was

cooled, and poured into 60 ml of 17% $NaHCO_3$. After 10 min., the aqueous solution was extracted with ether (2 x 50 ml). The combined ether solution was dried over $MgSO_4$, concentrated and chromatographed (60/40 ether/hexane) to afford 1.30 g (8.1 mmol, 85% yield) of 2 as a brown oil. An aliquot was distilled under the reduced pressure to give a colorless liquid for analysis.

Preparation of 3

To a flask charged with 342 mg (0.63 mmol) of $Hg(SCN)_2$ was added a premixed solution of I_2 in 8 ml of benzene. After 30 min. at room temperature (RT), to the mixture was added 202 mg (1.26 mmol) of 2 dissolved in 1 ml of benzene, and the flask containing the reaction mixture was wrapped with aluminum foil and stirred for 7.5 days at RT under argon atmosphere. The reaction mixture was then diluted with 20 ml of ether, washed with aqueous KI, $Na_2S_2O_3$, brine, dried over, and concentrated in vacuo. Flash column chromatography (1/1 ether/hexane) afforded 20 mg (0.06 mmol) of 3 (5% yield) as an oil along with 3 other isomers (11% yield).

Preparation of 4

To a flask charged with 21 mg (0.07 mmol) of 3 and 1 ml of benzene was added 0.05 ml (0.2 mmol, 3 eq.) of Bu_2SnH at RT. After 10 hr., the reaction mixture was treated with 35 mg of DBU (1,8-diazabicyclo[5,4,0]undec-7-ene) and 1 ml of wet ether. The resulting mixture was filtered off, concentrated and chromatographed (100% ether → 100% EtOAc) to afford 7.3 mg (0.03 mmol) of 4 (GHP 1073, 55% yield) as a white solid (mp; 123°C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09 (t, J = 3.2 Hz, 1H), 2.86 (s, 3H), 2.87-2.80 (m, 1H), 2.23-2.20 (m, 4H), 1.96-1.85 (m, 2H), 1.71-1.59 (m, 2H); FT=IR ($CHCl_3$) 3025 cm^{-1}, 2943, 2085, 1302.

Example 11

This example describes the synthesis of *exo*-2-(1′-hydroxyethyl)-5-isothiocyanatonorbornane (GHP 1075).

To a flask charged with 37.3 mg (0.19 mmol) of 1 (GHP 1067) and 1.5 ml of MeOH was added 11.0 mg (0.29 mmol) of $NaBH_4$ at 0°C. After 20 min., the excess $NaBH_4$ was quenched with a few drops of H_2O, diluted with either, dried over $MgSO_4$, and concentrated in vacuo. Preparative TLC (80/20 ether/hexane) afforded 21 mg (0.11 mmol) of 2 (GHP 1075, 56% yield) as a white solid (mixture of 2 disastereomers based on ^{1}H NMR).

^{1}H NMR (400 Mhz, $CDCl_3$) δ 3.58-3.54 (m), 3.49-3.45 (m), 3.39-3.32 (m), 2.54-2.46 (m), 2.18-2.16(d), 1.80-1.65 (m), 1.55-1.20 (m), 1.19 (d, J = 6.0 Hz); FT-IR ($CHCl_3$) 3613 cm^{-1}, 2966, 2872, 2097, 1343.

Example 12

This example describes the synthesis of 1-isothiocyanato-4-dimethylphosphonyl-butane (GHP 1078).

Preparation of 2

To a 25 ml flame dried round bottomed flask charged with 15.2 ml (45.6) mmol) of MeMgCl (Aldrich Chemical Co., 3.0 M in THF) was added 1.5 ml

(11.41 mmol) of diethyl phosphite 1 while the internal temperature was maintained around 25°C with occasional cooling with ice-water bath. After 1 hr, the mixture was cannulated into the flask charged with 2.55 ml (22.82 mmol) of dibromobutane and 15 ml of THF at 0°C under Ar atmosphere. Upon addition, the reaction mixture was heated under reflux for 5 hr, cooled, and dumped into 30 ml of cold dil. HCl. The resulting aqueous solution was extracted with $CHCl_3$ (3x50 ml), and the organic solution was washed with sat. k_2CO_3, dried over K_2CO_3, and concentrated in vacuo to give 2.48 g of crude product as a tan oil. Purification by flash column chromatography (silica-gel, 8/2 EtOAc/MeOH→6/4 EtOAc/hexane) afforded 0.72 g (3.42 mmol) of 2 as a colorless oil.

Preparation of 3

In a 100 ml round bottomed flask were placed 0.733 g (3.44 mmol) of 2, 0.766 g of potassium phthalimide and 20 ml of DMF. The mixture was heated under reflux for 4 hr, cooled and dumped into 60 ml of $CHCl_3$. The organic solution was washed with H_2O dried over $NaHCO_3$, and concentrated in vacuo to afford 0.92 g of 3 as a white solid (used for next reaction without further purification).

Preparation of 4

To a flask charged with 0.1 g of 3 was added 4 ml of methanolic hydrazine (0.2 M) at RT. After 14 hr at RT, the reaction mixture was concentrated, and the residue was treated with 5 ml of 1 N Hcl, washed with $CHCl_3$, strongly basified with solid NaOH. The basified solution was then extracted with $CHCl_3$ (2x20 ml),

and the combined organic solution was dried over K_2CO_3, and concentrated in vacuo to 33 mg (0.22 mmol) of 4 as a white solid (used for next reaction without further purification).

Preparation of 5 (GHP 1078)

To a flask charged with 33 mg (0.22 mmol) of 4 and 1 ml of $CHCl_3$ were added at RT 0.02 ml (0.27 mmol) of $CSCL_2$ and 0.3 ml of 1 N NaOH. After 35 min at RT, the reaction mixture was partitioned between 10 ml $CHCl_3$ of and 10 ml H_2O. The separated organic layer was dried over $MgSO_4$, concentrated in vacuo and chromatographed (silica-gel, 8/2 EtOAc/MeOH) to afford 29 mg (0.15 mmol) of 5 (GHP 1078) as a reddish yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 3.54 (t, J = 6.0 Hz, 2H), 1.82-1.70 (m, 6H), 1.48 (s, 3H), 1.44 (s, 3H); FT-IR ($CHCl_3$) 2941 cm^{-1}, 2191, 2097, 1302, 1173; ^{13}C NMR (400 MHz $CDCl_3$) δ44.5, 30.6 (d, J = 20.2 Hz, 1C), 30.7 (d, J = 34.7 Hz, 1C), 19.3, 16.2 (d, J = 69 Hz, 2C); ^{31}P NMR ($CDCl_3$) δ46.1; HRMS cald. for $C_3H_{14}NOPS$ 191.0534, found 195.0536.

Example 13

This example describes the synthesis of *cis*- or *trans*-3-(methylsulfonyl)cyclohexylmethylisothiocyanate (GHP 1079 or 1080).

Preparation of 2

2 was prepared according to the literature procedure, and the spectral data matched the literature values. Kozikowski, A.; Ames, A. *Tetrahedron* 1985, 4821.

Preparation of 3

To a 100 ml round bottomed flask were placed 0.438 g (3.0 mmol) of 2, 1.418 g of 2,4,6-triisopropylbenzenesulfonohydrazide (prepared according to literature procedure; Jirieny, J.; Orere, D.; Reese, C. *J. Chem. Soc. Perkin Trans. I*, 1980, 1487) and 8 ml of MeOH at RT. After 1 hr, 0.739 g of KCN was added at RT, and the resulting mixture was heated under gentle reflux for 3 hr. The reaction mixture was cooled, diluted with 20 ml of H_2O, and extracted with CH_2Cl_2 (2x20 ml). The organic solution was subsequently washed with aq. $NaHCO_3$, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatorgraph (8/2 hexane/EtOAc) to afford 0.360 g (2.3 mmol) of 3 (76 % yield) as a yellow oil.

Preparation of 4

To a flask charged with 0.36 g (2.32 mmol) of 3 and 10 ml of aqueous MeOH (9/1 v/v MeOH/H_2O) was added 2.75 g (4.64 mmol) of OXONE ($2KHSO_5 . KHSO_4 . K_2SO_4$) at RT. After 24 hr, the reaction mixture was filtered through a sintered glass funnel, and the filtered solid material was washed with 50 ml of $CHCl_3$. The combined organic solution was washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo to afford 0.246 g (1.31 mmol) of 4 (57 % yield) as a colorless oil. This material was used in the next reaction without purification.

Preparation of 5

To a $LiAlH_4$ suspension in 10 ml of anhydrous ether was cannulated 0.246 g (1.31 mmol) of 4 dissolved in 3 ml of THF at RT. Upon addition, the reaction

mixture was heated under reflux. After 2.5 hr, the reaction mixture was cooled, quenched with 0.5 ml of H_2O and 0.5 ml of 5% of NaOH, and filtered through a sintered glass funnel. The solid material filtered was thoroughly washed with ether. The combined organic solution was dried over K_2CO_3 and concentrated in vacuo to afford 0.15 g (0.78 mmol) of 5 (60% yield) as a colorless oil. This material was used in the next reaction without purification.

Preparation of 6a (GHP 1079) and 6b (GHP 1080)

To a flask charged with 0.15 g (0.78 mmol) of 5 and 3 ml of $CHCl_3$ were added 0.07 ml of $CSCl_2$ and 1.5 ml of 5% of NaOH at RT. After 1 hr, the reaction mixture was diluted with 10 ml of CH_2Cl_2, washed with H_2O and brine, dried over $MgSO_4$. concentrated in vacuo, and chromatographed (silica-gel, 1/1 hexane/EtOAc) to give 0.123 g (0.53 mmol) of products 67% yield) as a mixture 6a and 6b (1:1 ratio). HPLC (40/60 EtOAc/hexane) separation afforded GHP1079 and GHP 1080 (both as colorless oil). 6a (GHP 1079): ^{1}H NMR (400 MHz, $CDCl_3$) δ3.50 (d, J = 6.8 Hz, 2H), 3.09-3.03 (m, 1H), 2.88 (s, 3H), 2.45-2.37 (m, 1H), 2.14-2.07 (m, 1H), 1.98-1.84 (m, 4H), 1.74-1.66 (m, 1H), 1.59-1.41 (m, 2H); FT-IR ($CHCl_3$) 3013 cm^{-1}, 2943, 2872, 2191, 2097, 1449, 1308; ^{13}C NMR ($CDCl_3$) δ52.6, 43.5, 33.6, 28.0, 22.5, 22.1, 19.5, 14.9; HRMS calc. 233.0544 found 233.0545. 6b (GHP 1080): ^{1}H NMR (400 MHz, $CDCl_3$) δ3.47 (d, J = 6.0 Hz, 2H), 2.92-2.82 (m, 1H), 2.84 (s, 3H), 2.28-2.20 (m, 2H), 2.04 (tt, J = 6.8 Hz, 3.0, 1H), 1.87-1.75 (m, 2H), 1.53-1.27 (m, 3H), 1.06 (tq, J = 12.2 Hz, 3.6, 1H); FT-1R ($CHCl_3$) 3025 cm^{-1}, 2931, 2861, 2191, 2097, 1449,

1308; ^{13}C NMR ($CDCl_3$) δ56.5, 45.5, 32.6, 32.5, 23.9, 23.8, 20.0, 19.1; HRMS calc. 233.0544 found 233.0548.

Example 14

This example describes the synthesis of 6-isothiocyanato-2-hexanone ($CH_3CO(CH_2)_4NCS$)(GHP 1105).

Preparation of 2

To a flask charged with 2.252 g (19.22 mmol) of 1 and 20 ml of chloroform were added 40 ml of TMSCl. The mixture was heated around 45°C, then cooled to room temperature (RT). To this mixture was added 1.21 ml of CS_2 at RT, and the resulting solution was cooled to 0°C and treated with 8 ml of Et_3N. After 10 min, the reaction mixture was warmed to RT, and stirred for 2 hr, then cooled to 0°C, and treated with 2.0 ml of methyl chloroformate. After 45 min at 0°C, the reaction mixture was armed at RT, diluted 75 ml of hexane, filtered off, and concentrated in vacuo. The residue was dissolved in 75 ml of THF at 0°C, then 7 ml of H_2O was added to it. After 1.5 days, the reaction was dried over $MgSO_4$, concentrated and chromatographed (silica-gel, 20/80 EtOAc/hexane→1/1 EtOAc/hexane) to afford 1.563 g (9.80 mmol) of 2.

Preparation of 3 (GHP 1105)

A mixture of 0.3 g (1.89 mmol) of 2, 0.18 ml of $SOCl_2$ and 10 ml of $CHCl_3$ was heated under reflux for 2 hr. Upon removal of solvent, the residue was redissolved with 2 ml of dry ether. To this solution was added Me_2CuLi (prepared from CuI and 2MeLi in ether) at -78°C. After 2 hr at -78°C, the

reaction mixture was quenched with sat. NH_4Cl, warmed to RT, and extracted with ether. The organic solution was dried over $MgSO_4$, concentrated in vacuo, and chromatographed (silica-gel, 30/70 ether/hexane) to afford 0.022 g (0.13 mmol) of 3 (7% yield from 2) as an oil. ^1H NMR (300 MHz, $CDCl_3$) δ3.55-3.49 (m, 2H0, 2.53-2.47 (m, 2H0, 2.16 (s, 3H), 1.72-1.68 (m, 4H0; FT-IR ($CHCl_3$) 3019 cm^{-1}, 2191, 2112, 1715, 1224.

Example 15

This example describes the synthesis of 6-isothiocyanato-2-hexanol (GHP 1106).

To a flask charged with 15.0 mg (0.1 mmol) of 1 and 2 ml of EtOH was added 3.6 mg (0.1 mmol) of NaBH4 at 0°C. After 10 min, the reaction mixture was treated with 20 drops of H_20, dried over $MgSO_4$, concentrated in vacuo, and chromatographed (30/70 ether/hexane) to afford 7.5 mg (0.05 mmol) of 2 (GHP 1106, 49% yield) as a liquid.

^1H NMR (400 MHz, $CDCl_3$) δ3.87-3.77 (m, 1H), 3.53 (t, J = 6.6 Hz, 2H), 1.78-1.68 (m, 2H), 1.50-1.35 (m, 4H), 1.21 (d, 6.2 Hz, 3H); FT-IR ($CHCl_3$) 3615 cm^{-1}, 2945, 2180, 2107, 1375.

Example 16

This example describes the relative inducer activity of a large variety of sulforaphane analogs.

Analogs were synthesized and tested in the hepatoma cell assay described in Example 1. The results are shown in Table 3.

Table 3

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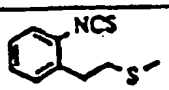
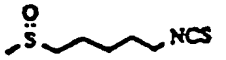
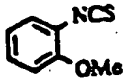
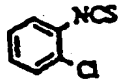
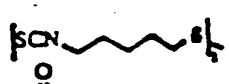
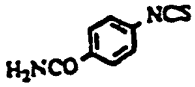
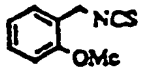
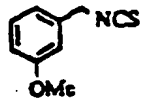
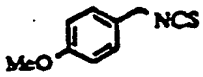
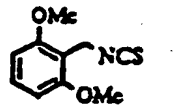
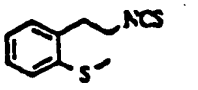
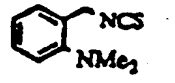
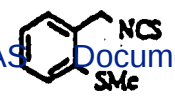
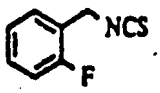
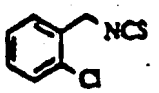
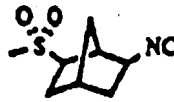
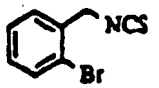
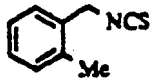
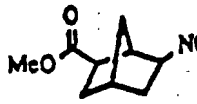
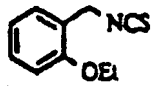
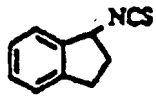
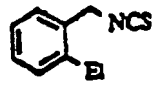
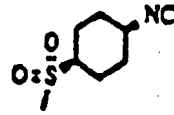
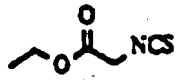
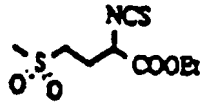
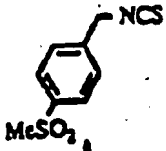
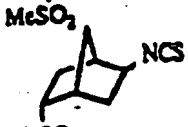
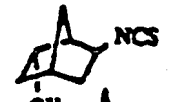
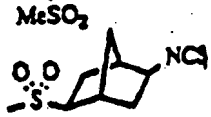
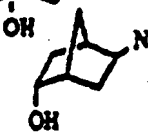
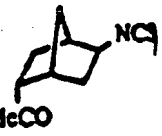
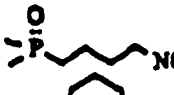
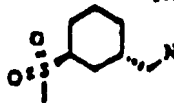
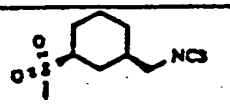
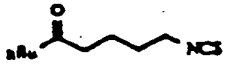
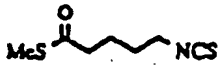
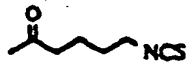
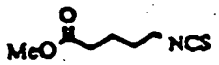
GHP 1001		1.71	GHP 1023		100
GHP 1002		0.94	GHP 1031		100
GHP 1003		0.98	GHP 1032		100
GHP 1004		0.83	GHP 1033		100
GHP 1005	 sulforaphane	0.20			
GHP 1006		2.30	GHP 1041		2.41
GHP 1007		0.82	GHP 1042		8.65
GHP 1008		3.52			
GHP 1009		2.36	GHP 1043		25
GHP 1010		1.32			
GHP 1021		4.3	GHP 1044		5.8
GHP 1022		7.4			
			GHP 1045		12.5

GHP 1046		6.8			
GHP 1047		13.1	GHP 1067		0.43
GHP 1048		14.1	GHP 1068		0.15
GHP 1049		12.5	GHP 1069		0.68
GHP 1050		3.7	GHP 1070		1.6
GHP 1051		2.5	GHP 1071		0.59
GHP 1052		38.9	GHP 1072		1.05
GHP 1053		12.5	GHP 1073		0.44
GHP 1061		8.2	GHP 1074		2.64
GHP 1062		1.2	GHP 1075		0.45
GHP 1063		1.02	GHP 1076		1.10
GHP 1064		0.66	GHP 1077		1.85
GHP 1065		0.77	GHP 1078		0.43
			GHP 1079		0.48

GHP 1080		0.41	GHP 1103		2.19
GHP 1081		2.0	GHP 1104		2.8
GHP 1101		1.97	GHP 1105		0.23
GHP 1102		2.81	GHP 1106		0.35

1. A pharmaceutical composition for cancer prevention comprising an active ingredient which is sulforaphane ((-)1-isothiocyanato-(4R)-(methylsulfinyl)butane) (CAS 4478-93-7) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon atoms linking said first and said second moieties, and wherein said analogue contains no pyridyl moieties.

2. The pharmaceutical composition of claim 1 wherein said analogue is not a heteroaromatic compound.

3. The pharmaceutical composition of claim 1 wherein said analogue is not an arylalkyl compound.

4. The pharmaceutical composition of claim 1 wherein said second moiety is a polar functional group selected from the group consisting of a carboxylic ester, a carboxylic acid, an ether, a halogen, a hydroxyl, a ketone, a nitrile, a nitro, a phosphine oxide, a sulfide, sulfone, a sulfoxide, a thioether, and a thioester.

5. The pharmaceutical composition of claim 1 wherein said second moiety is a polar functional group selected from the group consisting of a hydroxyl, a ketone, a phosphine oxide, a sulfone, and a sulfoxide.

6. The pharmaceutical composition of claim 1 wherein said chain of carbon atoms has at least three carbon atoms.

7. The pharmaceutical composition of claim 1 wherein said chain of carbon atoms has from three to five carbon atoms.

8. The pharmaceutical composition of claim 1 wherein said analogue is an olefin.

9. The pharmaceutical composition of claim 1 wherein said analogue is aliphatic.

10. The pharmaceutical composition of claim 1 wherein said chain of carbon atoms is part of a non-aromatic ring.

11. The pharmaceutical composition of claim 1 wherein said active ingredient is sulforaphane.

12. The pharmaceutical composition of claim 1 wherein said active ingredient is sulforaphene (4-isothiocyanato-(1R)-(methylsulfinyl)-1-(E)-butene) (CAS 2404-46-8).

13. The pharmaceutical composition of claim 1 wherein said active ingredient is selected from the group consisting of: 6-isothiocyanato-2-hexanon (GHP 1105); *exo*-2-acetyl-6-isothiocyanatonorbornane (GHP 1066); *exo*-2-isothiocyanato-6-methylsulfonylnorbornane (GHP 1068); 6-isothiocyanato-2-hexanol (GHP 1106); 1-isothiocyanato-4-dimethylphosphonylbutane (GHP 1078); *exo*-2-(1'-hydroxyethyl)-5-isothiocyanatonorbornane (GHP 1075); *exo*-2-acetyl-5-isothiocyanatonorbornane (GHP 1067); 1-isothiocyanato-5-methylsulfonylpentane (GHP 1003); and *cis*- or *trans*-3-(methylsulfonyl)cyclohexylmethylisothiocyanate (GHP 1079 or 1080).

14. The pharmaceutical composition of claim 1 further comprising a pharmaceutically acceptable excipient.

15. A compound which has cancer chemoprotection activity consisting of: 1-isothiocyanato-5-methylsulfonylpentane (CH_3-SO_2-$(CH_2)_5$-NCS) ((GHP 1003).

16. A compound which has cancer chemoprotection activity consisting of: 6-isothiocyanato-2-hexanone ($CH_3CO(CH_2)_4NCS$) (GHP 1105).

17. A compound which has cancer chemoprotection activity consisting of: *exo*-2-acetyl-6-isothiocyanatonorbornane (GHP 1066).

18. A compound which has cancer chemoprotection activity consisting of: *exo*-2-isothiocyanato-6-methylsulfonylnorbornane (GHP 1068).

19. A compound which has cancer chemoprotection activity consisting of: 6-isothiocyanato-2-hexanol (GHP 1106).

20. A compound which has cancer chemoprotection activity consisting of: 1-isothiocyanato-4-dimethylphosphonylbutane (GHP 1078).

21. A compound which has cancer chemoprotection activity consisting of: *exo*-2-(1'-hydroxyethyl)-5-isothiocyanatonorbornane (GHP 1075).

22. A compound which has cancer chemoprotection activity consisting of: *exo*-2-acetyl-5-isothiocyanatonorbornane (GHP 1067).

23. A compound which has cancer chemoprotection activity consisting of: *cis*- or *trans*-3-(methylsulfonyl)cyclohexylmethylisothiocyanate (GHP 1079 or 1080).

24. A method for protecting against cancer induction or progression, comprising:

administering to a mammal a chemoprotective composition consisting essentially of sulforaphane ((-)1-isothiocyanato-(4R)-(methylsulfinyl)butane) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate functionality and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon atoms linking said first and said second moieties, and wherein said analogue contains no pyridyl moieties, in an amount effective in producing a cancer preventive effect.

25. A food product which has been supplemented with an active chemoprotective compound, wherein said compound is sulforaphane ((-)1-isothiocyanato-(4R)-(methylsulfinyl)butane) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate functionality and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon atoms linking said first and said second moieties.

FIGURE 1

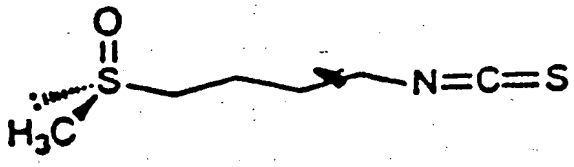


FIGURE 2

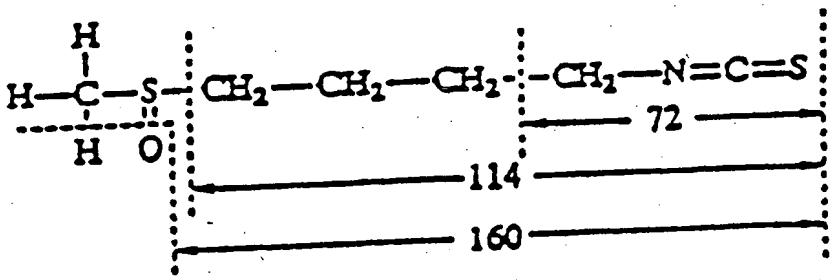


FIGURE 3

SYNTHESIS OF GHP 1003

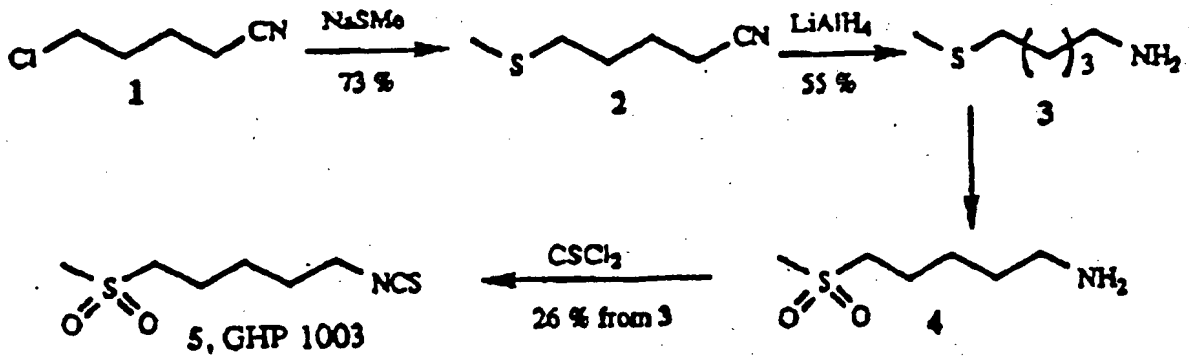


FIGURE 4

SYNTHESIS OF GHP 1066 AND GHP 1067

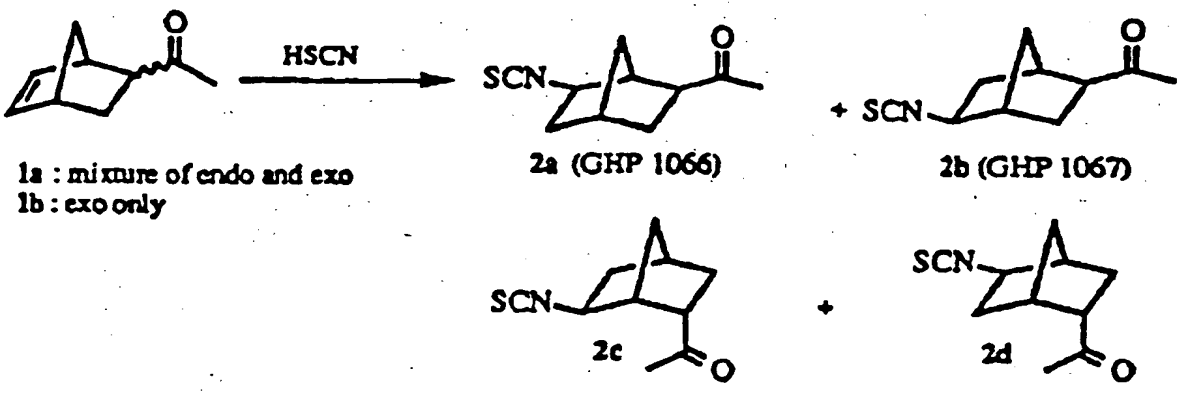


FIGURE 5

SYNTHESIS OF GHP 1064 AND GHP 1068

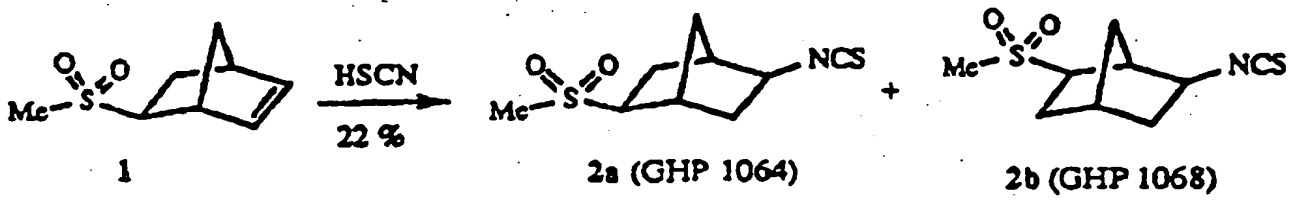


FIGURE 6

SYNTHESIS OF GHP 1073

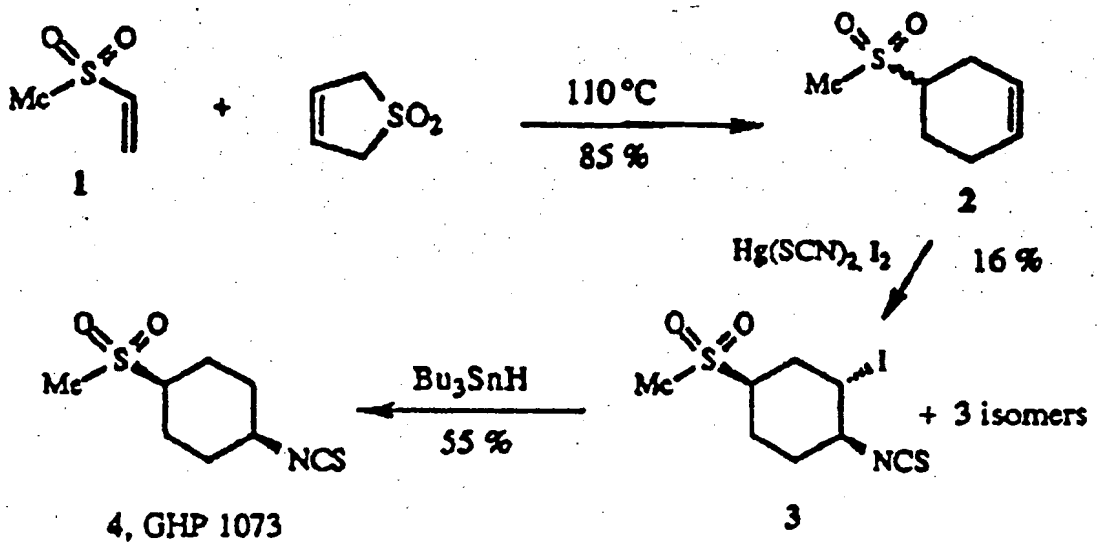


FIGURE 7

SYNTHESIS OF GHP 1075

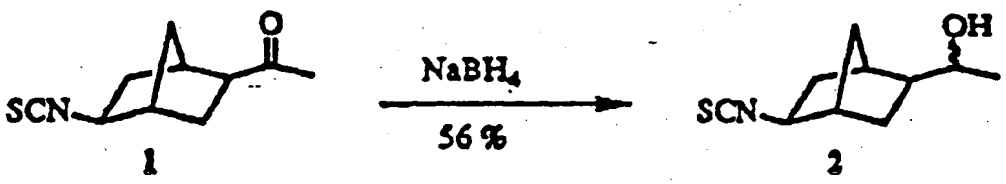


FIGURE 8

SYNTHESIS OF GHP 1078

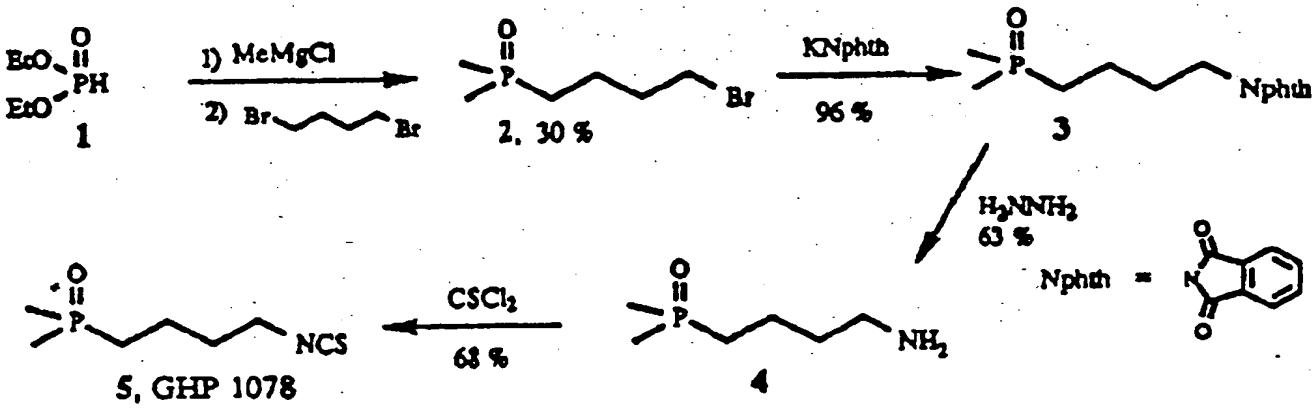


FIGURE 9

SYNTHESES OF GHP 1079 AND 1080

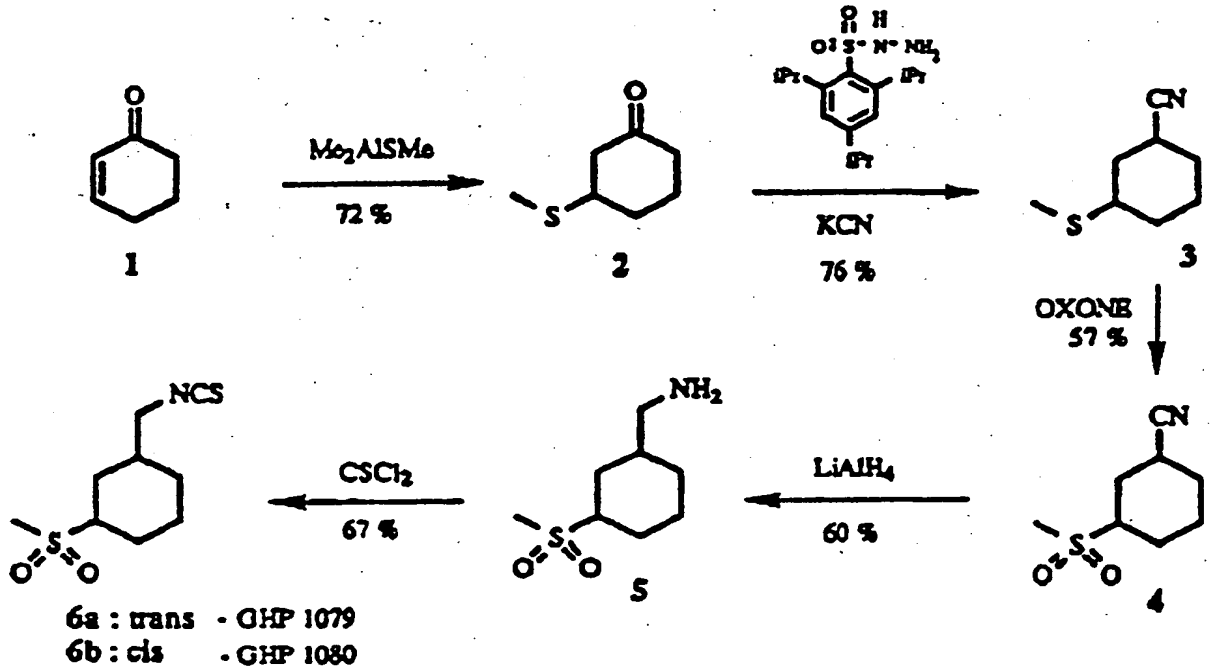


FIGURE 10

SYNTHESIS OF GHP 1105

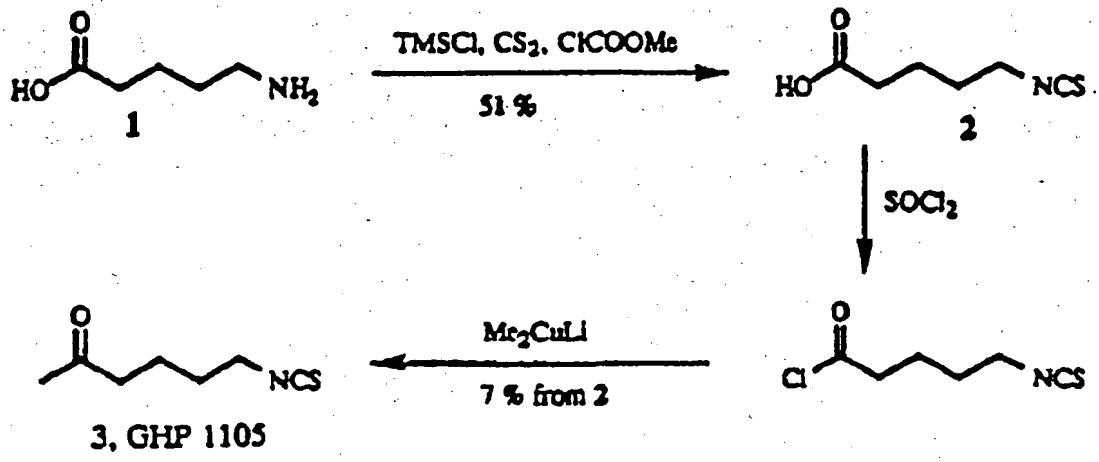
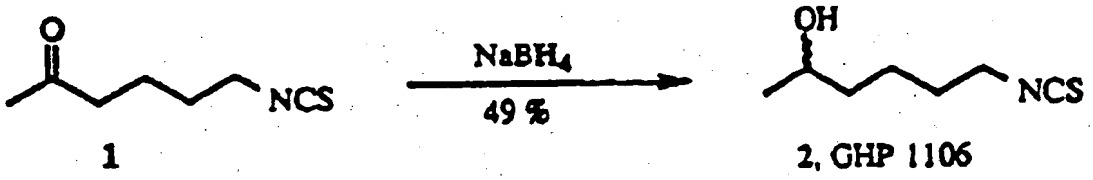


FIGURE 11

SYNTHESIS OF GHP 1106



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02453

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A01N 47/40; C07C 331/04, 331/12
US CL :514/514; 558/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/514; 558/17; an IPC(5): A01N 47/40; C07C 331/04, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
A	Carcinogenesis, Volume 8, No. 12, issued 1987, Lee W. Wattenberg, "Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine of benzi[a] pyrene on pulmonary and forestomach neoplasia in A/J mice", pages 1971-1973.	1-25
A	Cancer Research, Volume 51, issued 13 April 1991, Gary D. Stoner et al, "Inhibitory Effects of Phenethyl Isothiocyanate on N-Nitrosbenzylmethylamine Carcinogenesis in the Rat Esophagus", pages 2063-2068.	1-25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
17 MAY 1994

Date f mailing of the international search report
MAY 31 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

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Form PCT/ISA/210 (second sheet)(July 1992)*

Atty. Dkt. No. 046585/0138

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: **Jed FAHEY, *et al.***

Title: ***Cancer Chemoprotective Food Products***

Appl. No.: **09/825,989**

Filing Date: **April 5, 2001**

Examiner: **Cybille Delacroix-Muirheid**

Art Unit: **1614**

AMENDMENT TRANSMITTAL

Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

[X] Small Entity status under 37 C.F.R. § 1.9 and § 1.27 has been established by a Small Entity statement previously submitted.

[ ] Small Entity statement is enclosed.

[X] The fee required for additional claims is calculated below:

	Claims as Amended		Previously Paid For		Extra Claims Present		Rate		Additional Claims Fee
Total Claims:	24	□	20	=	4	x	$18.00	=	$72.00
Independents:	2	□	3	=	0	x	$84.00	=	$0.00
First presentation of any Multiple Dependent Claims:						+	$280.00	=	$0.00
							CLAIMS FEE TOTAL:	=	$72.00

[X] Applicant hereby petitions for an extension of time under 37 C.F.R. §1.136(a) for the total number of months checked below:

[ ]	Extension for response filed within the first month:	$110.00	$0.00
[X]	Extension for response filed within the second month:	$400.00	$400.00
[ ]	Extension for response filed within the third month:	$920.00	$0.00
[ ]	Extension for response filed within the fourth month:	$1,440.00	$0.00
[ ]	Extension for response filed within the fifth month:	$1,960.00	$0.00
		EXTENSION FEE TOTAL:	$400.00
		CLAIMS AND EXTENSION FEE TOTAL:	$472.00
[X]		Small Entity Fees Apply (subtract ½ of above):	$236.00
		TOTAL FEE:	$236.00

[ ] Please charge Deposit Account No. 19-0741 in the amount of $236.00. A duplicate copy of this transmittal is enclosed.

[X] A check in the amount of $236.00 is enclosed.

[X] The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date October 22, 2002

By _____

Richard C. Peet
Attorney for Applicant
Registration No. 35,792

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PATENT TRADEMARK OFFICE
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Atty. Dkt. No. 46585/138

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: **Jed FAHEY, et al.**

Title: **CANCER CHEMOPROTECTIVE FOOD PRODUCTS**

Appl. No.: **09/825,989**

Filing Date: **April 5, 2001**

Examiner: **Delacroix Muirhei, C.**

Art Unit: **1614**

AMENDMENT AND REPLY UNDER 37 C.F.R. §1.111

Commissioner for Patents
Washington, D.C. 20231

Sir:

In reply to the Office Action dated May 22, 2002, the due date for response having been extended two months to October 22, 2002, Applicant(s) submit(s) the following Amendment and Reply under 37 C.F.R. § 1.111.

Applicants concurrently file herewith a Petition for Extension of Time under 37 C.F.R. § 1.136(a), with provision for the required fee, to extend the period for response for two month(s) up to, and including, October 22, 2002. If additional fees are necessary to prevent abandonment of this application, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741.

IN THE CLAIMS:

In accordance with 37 C.F.R. § 1.121, please substitute for claims 58, 59, 61 and 62 the following rewritten version of the same claims, as amended. The changes are shown explicitly in the attached "Version with Markings to Show Changes Made".

58. (Amended) A method of making a food product comprising extracting glucosinolates and isothiocyanates from plant tissue having a high concentration of

glucosinolates, recovering said glucosinolates and isothiocyanates and adding said glucosinolates and isothiocyanates to food;

wherein said extracting comprises contacting said plant tissue with a non-toxic solvent at a temperature sufficient to inactivate myrosinase enzyme activity.

59. (Amended) The method according to claim 58, wherein said non-toxic solvent is water.

61. (Amended) The method according to claim 58, wherein said non-toxic solvent is liquid carbon dioxide.

62. (Amended) The method according to claim 58, wherein said non-toxic solvent is ethanol.

Please add the following newly added claims.

--68. (New) The method of claim 58 wherein said food product is selected from the group consisting of a bread, a drink, a soup, a salad, a sandwich and a cereal.

69. (New) The method of claim 68 wherein said drink is a tea.

70. (New) The method of claim 58 wherein said extracting further comprises homogenizing said plant tissue with said non-toxic solvent.

71. (New) The method of claim 63 wherein said sprouts, seeds, plants or plant parts have at least 250,000 units per gram fresh weight of Phase 2 enzyme-inducing potential. --

REMARKS

Status of the Claims

By this amendment, claims 58, 59, 61 and 62 are amended and claims 68-71 are added. Upon entry of this Amendment, claims 48-71 will be pending in the application.

Support for the amendments to claim 58 are found throughout the specification, for example on page 9, lines 1-18; page 11, lines 20-24; page 17, line 15; and page 21, lines 10-29. Support for newly added claim 68 is found in the specification on page 11, lines 24-31. Support for newly added claim 69 is found in the specification on page 11, line 29 and page 21, line 36, through page 22, line 2. Support for newly added claim 70 is found in the specification on page on page 36, lines 13-21. Support for claim 71 is found on page 20, line 29 of the specification. Claims 68-71 are added to further define claim scope.

Because the foregoing amendments do not introduce new matter, entry thereof by the examiner is respectfully requested.

Issues Under Information Disclosure Statement (IDS)

The Examiner states that Applicants' IDS received April 5, 2001 has been considered in part, i.e. U.S. Patents. However, the Examiner states that references A3-A76 were not in parent application 09/425,890 and requests copies of these documents. Attached herewith are copies of references A3-A46 and A48-A76. A copy of A47 will be provided in the near future. Also attached is a copy of the Form PTO-1449 submitted with the IDS filed on April 5, 2001. Applicants respectfully request that any listed document be considered by the Examiner and be made of record in the present application and that an initialed copy of the enclosed Form PTO-1449 be returned in accordance with MPEP § 609.

Claim Objections

Claim 53 is objected to by the Examiner because the claim contains an improper Markush group. Applicants have amended claim 53 by replacing "or" with --and--. Applicants respectfully request withdrawal of the rejection.

Claim Rejections - 35 U.S.C. § 112, Second Paragraph

Claims 59, 61 and 62 are rejected by the Examiner under 35 U.S.C. § 112, second paragraph for being indefinite. Applicants respectfully request reconsideration and withdrawal of the rejection.

The Examiner asserts that in claims 59, 61 and 62, there is insufficient antecedent basis for the term "said solvent" in line one. Applicants respectfully disagree because there is antecedent basis for the term "said solvent". However, in order to expedite prosecution, Applicants have amended claims 59, 61 and 62 to recite "said non-toxic solvent".

Allowable Subject Matter

The Examiner asserts that claims 48-57 are free from the prior art because the prior art does not disclose or fairly suggest Applicants' claimed method.

Claim Rejections - 35 U.S.C. § 102

A. Claims 58, 59, 62 and 63 are rejected by the Examiner under 35 U.S.C. § 102 as being anticipated by Jones et al. (U.S. Patent No. 5,158,656). Applicants respectfully request reconsideration and withdrawal of the rejection.

The present claims, as amended, are directed to a method of making a food product comprising extracting glucosinolates and isothiocyanates from plant tissue rich in glucosinolates, recovering the glucosinolates and isothiocyanates, and adding the glucosinolates and isothiocyanates to a food. The extracting step involves contacting said plant tissue with a non-toxic solvent at a temperature sufficient to inactivate myrosinase enzyme activity. Jones et al. is directed toward a process for producing a protein concentrate for a dehulled, defatted oleaginous thioglucoside and phenolic containing seed material. The two methods are different from one another because while the method of the present invention involves recovering glucosinolates and isothiocyanates and adding the recovered materials to food, the method of Jones et al. involves removing glucosinolates and other materials from oilseeds in order to recover a nutritious protein concentrate that is free of these materials. Therefore, the method of Jones et al. comprises completely different steps and results in a completely different product than the presently claimed method.

Furthermore, Jones et al. teaches away from the presently claimed method. The present specification teaches that most of the Phase 2 inducer potential of crucifer plants is due to their content of isothiocyanates and their biogenic precursors, glucosinolates. See page 15, lines 3-6.

Thus, the present method is directed to recovering glucosinolates and isothiocyanates and adding these compounds to food. In contrast, in column 1, lines 7-13 of Jones et al., it states that "certain oilseeds...contain thioglucosides (glucosinolates) which, by means of endogenic enzymes, e.g. myrosinases, are split into the deleterious substances isothiocyanates and/or oxazolidinethiones, and glucose and bisulphate." In column 3, lines 40-44, Jones et al. states "The glucosinolates contained in rapeseed are, as is well known, hydrolyzed by myrosinase under the appropriate conditions to isothiocyanates, nitriles and oxazolidinethiones some of which are known to cause goiter." Jones et al. also states at column 3, lines 48-53 that "it is essential for food use, to remove the glucosinolates and those other factors that can cause unattractive flavor and coloration and decreased nutritive value of foods." Therefore, the present invention is not anticipated by Jones et al. because the method of Jones et al. removes glucosinolates to yield a nutritious protein extract, while the present invention recovers the glucosinolates and adds them to food. Additionally, Jones et al. teaches away from the present invention.

B. Claims 58, 59 and 63 are rejected by the Examiner under 35 U.S.C. § 102 as being anticipated by Anjou et al. (U.S. Patent No. 4,083,836). Applicants respectfully request reconsideration and withdrawal of the rejection.

The method of Anjou et al. is directed toward preparing an edible protein concentrate which is non-toxic and has an acceptable light color and a neutral bland flavor. As discussed above with respect to Jones et al., the method of Anjou et al. differs from the presently claimed method because while the method of the present invention involves recovering glucosinolates and isothiocyanates and adding the recovered materials to food, the method of Anjou et al. involves removing glucosinolates and other materials from seeds of Brassica species in order to recover a nutritious protein concentrate that is free of these materials. Therefore, the method of Anjou et al. comprises completely different steps and results in a completely different product than the presently claimed method.

Furthermore, as discussed above with respect to Jones et al., Anjou et al. teaches away from the presently claimed method. Anjou et al. emphasizes the necessity of removing

glucosinolates from seeds of Brassica species in order to produce a protein concentrate "which is non-toxic, has an acceptable light color, a neutral and mild flavor and a high nutritional value and which thus is well suited for human consumption." See column 1, lines 8-11. In column 1, lines 21-28, Anjou et al. discusses the drawbacks of prior art oil extractions which contained "glucosinolates, which could be split into deleterious compounds with pungent flavor." Anjou et al. therefore teaches away from the present method by emphasizing the necessity of removing glucosinolates from Brassica seeds in order to produce a non-toxic protein concentrate that is suited for human consumption. Therefore, the present invention is not anticipated by Anjou et al. because the method of Anjou et al. removes glucosinolates to yield a nutritious protein extract that is free of glucosinolates, while the present invention recovers the glucosinolates and adds them to food. Additionally, Anjou et al. teaches away from the present invention.

Claim Rejections - 35 U.S.C. § 103

Claim 60 is rejected by the Examiner under 35 U.S.C. § 103 as being unpatentable over Anjou et al. The Examiner asserts that while Anjou et al. does not disclose that the temperature of the leach water is 100° C, it would have been obvious to one of ordinary skill in the art to further modify the leaching method of Anjou et al. such that the temperature is effective to result in optimum extraction of glucosinolates from the seed material, since Anjou et al. establish that the glucosinolate leaching process is temperature dependent. Applicants respectfully request reconsideration and withdrawal of the rejection.

A proper rejection for obviousness under §103 requires consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition, or device, or carry out the claimed process and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure. [emphasis added] *In re Vaeck*, 947 F.2d 488, 493, 20 USPQ2d 1438 (Fed. Cir. 1991).

In the pending case, the examiner has failed to establish a *prima facie* case of obviousness. As discussed above, Anjou et al. teaches away from the present. The present specification teaches that most of the Phase 2 inducer potential of crucifer plants is due to their content of isothiocyanates and their biogenic precursors, glucosinolates. See page 15, lines 3-6. Thus, the present method is directed to recovering glucosinolates and isothiocyanates and adding these compounds to food. In contrast, the method of Anjou et al. involves removing glucosinolates to produce a protein concentrate that is suitable for human consumption. Anjou et al. emphasizes the necessity of removing glucosinolates from seeds of Brassica species in order to produce a protein concentrate "which is non-toxic, has an acceptable light color, a neutral and mild flavor and a high nutritional value and which thus is well suited for human consumption." See column 1, lines 8-11. In column 1, lines 21-28, Anjou et al. discusses the drawbacks of prior art oil extractions which contained "glucosinolates, which could be split into deleterious compounds with pungent flavor." Because Anjou et al. teaches a method that is completely opposite to the teachings of the present specification, Anjou et al. would not have suggested to those of ordinary skill in the art that they should carry out the claimed process. Additionally, Anjou et al. would not have provided a reasonable expectation of success in carrying out the method of the present invention because Anjou et al. emphasizes the necessity of removing glucosinolates from seeds of Brassica-species because of their potentially toxic breakdown products and pungent flavor. Therefore, claim 60 is not obvious over Anjou et al.

Recent Decisions by the United States Court of Appeals for the Federal Circuit

In compliance with MPEP 2001.06(c), attached, as Exhibit 1, is a copy of a decision by the Court of Appeals for the Federal Circuit for In re Cruciferous Sprout Litigation. This decision has no impact on the validity of the claims presented herein. The prior art does not teach or suggest a method of making a food product comprising extracting glucosinolates and isothiocyanates from plant tissue.

CONCLUSION

As the above-presented amendments and remarks address and overcome all of the rejections presented by the examiner, withdrawal of the rejections and allowance of the claims are respectfully requested.

If the examiner has any questions concerning this application, he or she is requested to contact the undersigned.

Respectfully submitted,

Date October 22, 2002

By _____

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Attorney for Applicant
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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

58. (Amended) A method of making a food product comprising extracting glucosinolates and isothiocyanates from plant tissue [rich in] having a high concentration of glucosinolates, [with the exception of cabbage, cress, mustard and radish sprouts, comprising homogenizing said plant tissue in a non-toxic solvent at a temperature sufficient to inactivate myrosinase enzyme activity] recovering said glucosinolates and isothiocyanates and adding said glucosinolates and isothiocyanates to food;

wherein said extracting comprises contacting said plant tissue with a non-toxic solvent at a temperature sufficient to inactivate myrosinase enzyme activity.

59. (Amended) The method according to claim 58, wherein said non-toxic solvent is water.

61. (Amended) The method according to claim 58, wherein said non-toxic solvent is liquid carbon dioxide.

62. (Amended) The method according to claim 58, wherein said non-toxic solvent is ethanol.

1614 2282

Atty. Dkt. No 046585/0138

#6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: **Jed FAHEY, *et al.***

Title: ***Cancer Chemoprotective Food Products***

Appl. No.: **09/825,989**

Filing Date: **April 5, 2001**

Examiner: **Cybille Delacroix-Muirheid**

Art Unit: **1614**

PETITION FOR EXTENSION OF TIME

Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant hereby petitions the Commissioner under 37 C.F.R. 1.136(a) for a two-month extension of time for response in the above-identified application for the period required to make the attached response timely.

The extension fee for response within the second month is $200.00. A check for this amount is enclosed herewith.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Respectfully submitted,

Date October 22, 2002

By _____

Richard C. Peet
Attorney for Applicant
Registration No. 35,792

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Source: Legal > Area of Law - By Topic > Patent Law > Cases and Court Rules > **Patent Cases from Federal Courts**
Terms: **in re cruciferous sprout litigation** (Edit Search)

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*301 F.3d 1343, *; 2002 U.S. App. LEXIS 17185, ***

IN RE CRUCIFEROUS SPROUT LITIGATION; BRASSICA PROTECTION PRODUCTS LLC and JOHNS HOPKINS UNIVERSITY, Plaintiffs-Appellants, v. SUNRISE FARMS, BECKY CRIKELAIR, and FRANK CRIKELAIR, Defendants-Appellees, and EDRICH FARMS INC., EDWARD B. STANFIELD, III, EDWARD F. STANFIELD, JR., RICHARD STANFIELD, and SALLY F. STANFIELD, Defendants-Appellees, and BANNER MOUNTAIN SPROUTS, BANNER MOUNTAIN SPROUTS INC., and LAWRENCE RAVITZ, Defendants-Appellees, and HARMONY FARMS, GREG LYNN, and LORNA LYNN, and INTERNATIONAL SPECIALTY SUPPLY and ROBERT L. RUST, Defendants-Appellees.

02-1031

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

301 F.3d 1343; 2002 U.S. App. LEXIS 17185

August 21, 2002, Decided

PRIOR HISTORY: [1]** Appealed from: United States District Court for the District of Maryland. Judge William M. Nickerson.

In re Cruciferous Sprout Patent Litig., 168 F. Supp. 2d 534 (D. Md. 2001).

DISPOSITION: Affirmed.

CASE SUMMARY

PROCEDURAL POSTURE: Plaintiffs, patent owners, had three patents-in-suit related to the growing an eating of sprouts to reduce the level of carcinogens in animals. The United States District Court for the District of Maryland granted defendants, farmers', motion for summary judgment on the grounds the patents were invalid as anticipated by the prior art. The patent owners appealed.

OVERVIEW: The patents described methods of preparing food products that contained high levels of substances that induce Phase 2 enzymes. These enzymes were part of the human body's mechanism for detoxifying potential carcinogens. Thus, they had a chemoprotective effect against cancer. The patent owners filed patents related to the method of preparing food rich in glucosinolate, preparing human food products from sprouts, and method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, as well as a method of reducing the level of carcinogens in a mammal, by creating a "food product" from sprouts and then administering said food product to a mammal. The court of appeals held that it rejected the patent owner's proposed claim construction for the phrases "rich in glucosinolates" and "high in Phase 2 enzyme-inducing potential" because it was inconsistent with the language of the dependant claims. The patent owners had not done anything more than recognize properties inherent in certain prior art sprouts, and had not invented anything new, and therefore, their claims were invalid.

OUTCOME: The judgment was affirmed.

CORE TERMS: sprout, patent, phase, glucosinolate, rich, enzyme-inducing, seed, food product, specification, cruciferous, preamble, invention, anticipated, disclose, plant, alloy, food, kale, summary

judgment, preparing, broccoli, cabbage, enzyme, chemoprotective, harvesting, cultivars, inventor, cancer, eating, anticipation

CORE CONCEPTS - ◆ Hide Concepts

Civil Procedure > Appeals > Standards of Review > De Novo Review

An appellate court reviews a grant of summary judgment de novo, drawing all reasonable factual inferences in favor of the non-moving party.

Patent Law > Infringement > Summary Judgment

With regard to a patent infringement claim, summary judgment is appropriate when there is no genuine issue of material fact and the moving party is entitled to judgment as a matter of law. Anticipation is a question of fact, and is determined by first construing the claims and then comparing the properly construed claims to the prior art.

Civil Procedure > Appeals > Standards of Review > De Novo Review

Patent Law > Jurisdiction & Review > Standards of Review

Under patent law, claim construction is an issue of law that an appellate court reviews de novo. An appellate court also determines de novo whether the evidence in the record raises any genuine disputes about material facts.

Patent Law > Specification & Claims > Claim Preambles

Under patent law, no litmus test defines when a preamble limits claim scope. Whether to treat a preamble as a limitation is a determination resolved only on review of the entirety of the patent to gain an understanding of what the inventors actually invented and intended to encompass by the claim. In general, a preamble limits the claimed invention if it recites essential structure or steps, or if it is "necessary to give life, meaning, and vitality" to the claim. Clear reliance on the preamble during prosecution to distinguish the claimed invention from the prior art may indicate that the preamble is a claim limitation because the preamble is used to define the claimed invention.

Patent Law > Infringement > Claim Interpretation

Under patent law, the words of a claim are generally given their ordinary and accustomed meaning, unless it appears from the specification or the file history that they were used differently by the inventor. However, limitations appearing in the specification will not be read into claims, and interpreting what is meant by a word in a claim is not to be confused with adding an extraneous limitation appearing in the specification, which is improper.

Patent Law > Infringement > Claim Interpretation

Under patent law, a violation of the doctrine of claim differentiation is found when a proposed construction would render another claim superfluous.

Patent Law > Novelty & Anticipation

Under patent law, in order to prove that a claim is anticipated under 35 U.S.C.S. § 102(b), a defendant must present clear and convincing evidence that a single prior art reference discloses, either expressly or inherently, each limitation of the claim.

Patent Law > Novelty & Anticipation

Under patent law, a prior art reference may anticipate when the claim limitations not expressly found in that reference are nonetheless inherent in it. Under the principles of inherency, if the prior art necessarily functions in accordance with, or includes, the claimed limitations, it anticipates. Inherency is not necessarily coterminous with the knowledge of those of ordinary skill in the art. Artisans of ordinary skill may not recognize the inherent characteristics or functioning of the prior art.

Patent Law > Patentable Subject Matter > Products

Under patent law, the basic provision of 35 U.S.C.S. § 101, provides in relevant part that whoever invents or discovers any new composition of matter, or any new improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Patent Law > Novelty & Anticipation

Under patent law, newly discovered results of known processes directed to the same purpose are not patentable because such results are inherent.

Patent Law > Novelty & Anticipation

Under patent law, the public remains free to make, use, or sell prior art compositions or processes, regardless of whether or not they understand their complete makeup of the underlying scientific principles which allow them to operate.

COUNSEL: E. Anthony Figg, Rothwell, Figg, Ernst & Manbeck, P.C., of Washington, DC, argued for plaintiffs-appellants. With him on the brief were Joseph A. Hynds and Mark I. Bowditch.

Joseph A. Kromholz, Ryan, Kromholz & Manion, of Milwaukee, Wisconsin, argued for defendants-appellees. With him on the brief for defendants-appellees Sunrise Farms, et al. was Daniel R. Johnson. On the brief for defendants-appellees Harmony Farms, et al. was Delbert J. Barnard, Barnard & Pauly, P.S. On the brief for defendants-appellees Edrich Farms Inc., et al. was Philip M. Andrews, Kramon & Graham, P.A., of Baltimore, Maryland. On the brief for defendants-appellees Banner Mountain Sprouts, et al. was Donald W. Ullrich, Jr., The Ullrich Law Firm, of Sacramento, California.

JUDGES: Before CLEVENGER, BRYSON, and PROST, Circuit Judges.

OPINIONBY: PROST

OPINION:

[*1345] PROST, Circuit Judge.

Brassica Protection Products LLC and Johns Hopkins University (collectively "Brassica") appeal from the decision of the United States District Court for the District of Maryland granting summary judgment that U.S. Patent **[**2]** Nos. 5,725,895 ("the '895 patent"), 5,968,567 ("the '567 patent"), and 5,968,505 ("the '505 patent") are invalid as anticipated by the prior art. In re Cruciferous Sprout Patent Litig., 168 F. Supp. 2d 534, 60 USPQ2d 1758 (D. Md. 2001). We affirm the district court's ruling.

BACKGROUND

The three patents-in-suit relate to growing and eating sprouts to reduce the level of carcinogens in animals, thereby reducing the risk of developing cancer. Specifically, the patents describe methods of preparing food products that contain high levels of substances that induce Phase 2 enzymes. These enzymes are part of the human body's mechanism for detoxifying potential carcinogens. Thus, they have a chemoprotective effect against cancer. '895 patent, col. 1, ll. 28-34. Foods that are rich in glucosinolates, such as certain cruciferous sprouts, have high Phase 2 enzyme-inducing potential. The inventors of the patents-in-suit recognized that the Phase 2 enzyme-inducing agents (or their glucosinolate precursors) are far more concentrated in certain sprouts (such as broccoli and cauliflower but not cabbage, cress, mustard or radish) that are harvested before the two-leaf stage than **[**3]** in corresponding adult plants. Id. at col. 7, l. 63 - col. 8, l. 14. However, glucosinolate levels in cruciferous plants can be highly variable. See id. at col. 12, ll. 66-67 ("There is variation in inducer potential among different broccoli cultivars."). According to the inventors, it is therefore desirable to select the seeds of those cruciferous plants which, when germinated and harvested before the two-leaf stage, produce sprouts that contain high levels of the desired enzyme-inducing potential.

The '895 patent was filed on September 15, 1995, and claims, inter alia, "A method of preparing a food product rich in glucosinolates, comprising germinated cruciferous seeds, with the exception of cabbage, cress, mustard and radish seeds, and harvesting sprouts prior to the 2-leaf stage, to form a food product comprising a plurality of sprouts." '895 patent, claim 1. The '567 patent is a continuation of the '895 application and it claims a "method of preparing a human food product" from sprouts. '567 patent, claims 1 and 9. The '505 patent is a divisional of the '895 application and it claims a "method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, **[**4]** " as well as a "method of reducing the level of carcinogens in a mammal," by creating a "food product" from sprouts and then "administering said food product" to a mammal. '505 patent, claims 1 and 16.

The three patents-in-suit are owned by Johns Hopkins University and exclusively licensed to Brassica Protection Products LLC. Johns Hopkins and Brassica sued Sunrise Farms, Becky Crikelair, Frank Crikelair, Edrich Farms, Inc., Edward B. Stanfield, III, Edward F. Stanfield, Jr., Richard Stanfield, Sally F. Stanfield, Banner Mountain Sprouts, Banner Mountain Sprouts, Inc., Lawrence Ravitz, Harmony **[*1346]** Farms, International Specialty Supply, Greg Lynn, Lorna Lynn and Robert L. Rust (collectively "defendants") in various district courts. Pursuant to 28 U.S.C. § 1407, the Judicial Panel on Multidistrict Litigation consolidated the various cases in the District of Maryland for pretrial proceedings. On June 7, 2001, the defendants filed a joint motion for partial summary judgment of invalidity, arguing that the patents were anticipated by prior art references disclosing growing and eating sprouts. Brassica filed a cross-motion for summary judgment that the patents are not **[**5]** invalid. On July 23, 2001, the district court held a Markman hearing to address claim construction issues and the parties' motions for summary judgment.

On August 10, 2001, the court granted defendants' motion for summary judgment of invalidity and denied Brassica's cross-motion for summary judgment. According to the district court, "the record before the Court makes it abundantly clear that, prior to the issuance of the patents-in-suit, one skilled in the art could, by following the teachings of the prior art, germinate broccoli seeds, harvest the sprouts, and sell them as a food product." In re Cruciferous Sprout Patent Litig., 168 F. Supp. 2d at 540, 60 USPQ2d at 1762. While recognizing that the inventors of the patents-in-suit may have discovered a new and significant property of certain types of cruciferous sprouts, the district court concluded that "merely describing unexpected beneficial results of a known process does not entitle Plaintiffs to patent that process." Id. at 538, 60 USPQ2d at 1760. Thus, a "plant (broccoli sprouts), long well known in nature and cultivated and eaten by humans for decades, [cannot] be patented merely on **[**6]** the basis of a recent realization that the plant has always had some heretofore unknown but naturally occurring beneficial feature." Id. at 537, 60 USPQ2d at 1759. On October 1, 2001, the court entered a Judgment Under Rule 54(b) in favor of defendants but limited its invalidity ruling to claims 1-6 and 9 of the '895 patent, claims 1-8 of the '567 patent, and claims 1 and 16 of the '505 patent. In re Cruciferous Sprout Patent Litig., MDL Docket No. 1388 (D. Md. Oct. 1, 2001) (Rule 54(b) Determination). Brassica appeals the judgment of invalidity, arguing that the district court failed to properly construe the claims and did not apply the properly construed claims to the prior art when determining that the claims are anticipated under 35 U.S.C. § 102(b). We have jurisdiction under 28 U.S.C. § 1295 (a)(1).

DISCUSSION

This court reviews a grant of summary judgment de novo, drawing all reasonable factual inferences in favor of the non-moving party. See, e.g., Anderson v. Liberty Lobby, Inc., 477 U.S. 242, 255, 91 L. Ed. 2d 202, 106 S. Ct. 2505 (1986). Summary judgment is appropriate when there **[**7]** is no genuine issue of material fact and the moving party is entitled to judgment as a matter of law. Id. at 247-48. Anticipation is a question of fact, Gen. Elec. Co. v. Nintendo Co., 179 F.3d 1350, 1353, 50 USPQ2d 1910, 1912 (Fed. Cir. 1999), and is determined by first construing the claims and then comparing the properly construed claims to the prior art, Gechter v. Davidson, 116 F.3d 1454, 1457, 43 USPQ2d 1030, 1032 (Fed. Cir. 1997). Claim construction is an issue of law that we review de novo. Cybor Corp. v. FAS Techs., Inc., 138 F.3d 1448, 1456, 46 USPQ2d 1169, 1174 (Fed. Cir. 1998) (en banc). We also determine de novo whether the evidence in the record raises any genuine disputes about material facts. Gen. Elec., 179 F.3d at 1353, 50 USPQ2d at 1912.

[*1347] I.

Brassica contends that the district court erroneously construed the claims by failing to treat the preamble of claim 1 of the '895 patent as a limitation of the claims. In addition, Brassica argues that the district court failed to construe the limitations "rich in glucosinolates" (appearing in claims 1 and 9 of the '895 patent) and [**8] "high Phase 2 enzyme-inducing potential" (appearing in claim 1 of the '567 patent and claims 1 and 16 of the '505 patent).

No litmus test defines when a preamble limits claim scope. Corning Glass Works v. Sumitomo Elec. U.S.A., Inc., 868 F.2d 1251, 1257, 9 USPQ2d 1962, 1966 (Fed. Cir. 1989). Whether to treat a preamble as a limitation is a determination "resolved only on review of the entirety of the patent to gain an understanding of what the inventors actually invented and intended to encompass by the claim." Id.; Catalina Mktg. Int'l v. Coolsavings.com, Inc., 289 F.3d 801, 808, 62 USPQ2d 1781, 1785 (Fed. Cir. 2002). In general, a preamble limits the claimed invention if it recites essential structure or steps, or if it is "necessary to give life, meaning, and vitality" to the claim. Catalina Mktg., 289 F.3d at 808, 62 USPQ2d at 1784 (quoting Pitney Bowes, Inc. v. Hewlett-Packard Co., 182 F.3d 1298, 1305, 51 USPQ2d 1161, 1165 (Fed. Cir. 1999)). Clear reliance on the preamble during prosecution to distinguish the claimed invention from the prior art may indicate that the preamble is a claim limitation because [**9] the preamble is used to define the claimed invention. Catalina Mktg., 289 F.3d at 808, 62 USPQ2d at 1785; Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc., 246 F.3d 1368, 1375, 58 USPQ2d 1508, 1513 (Fed. Cir. 2001).

In this case, both the specification and prosecution history indicate that the phrase "rich in glucosinolates" helps to define the claimed invention and is, therefore, a limitation of claim 1 of the '895 patent. The specification, for example, states that "this invention relates to the production and consumption of foods which are rich in cancer chemoprotective compounds." '895 patent, col. 1, ll. 18-19. A stated object of the invention is "to provide food products and food additives that are rich in cancer chemoprotective compounds." Id. at col. 2, ll. 38-39. The specification therefore indicates that the inventors believed their invention to be making food products that are rich in chemoprotective compounds, or, in other words, food products "rich in glucosinolates."

n1 In addition, during reexamination n2 of the '895 patent the patentee argued as follows:

> Claim 1 of the patent, for example, is directed to "[a] method [**10] of preparing a [*1348] food product rich in glucosinolates, . . . and harvesting sprouts prior to the 2-leaf stage, to form a food product comprising a plurality of sprouts." . . . Although "rich in glucosinolates" is recited in the preamble of the claim, the pertinent case law holds that the preamble is given weight if it breathes life and meaning into the claim. . . . Accordingly, the cited prior art does not anticipate the claims because it does not explicitly teach a method of preparing a food product comprising cruciferous sprouts that are rich in glucosinolates or contain high levels of Phase 2 inducer activity.

This language shows a clear reliance by the patentee on the preamble to persuade the Patent Office that the claimed invention is not anticipated by the prior art. As such, the preamble is a limitation of the claims. See Bristol-Myers Squibb, 246 F.3d at 1375, 58 USPQ2d at 1513.

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n1 Phase 2 enzymes are part of the human body's mechanism for detoxifying potential carcinogens. These enzymes therefore have a chemoprotective effect against cancer. According to the '895 patent, "most of the [Phase 2 enzyme] inducer potential of crucifer plants is due to their content of isothiocyanates and their biogenic precursors, glucosinolates." '895 patent, col. 8, ll. 14-16. [**11]

n2 On December 6, 1999, the Patent Office granted a request for reexamination of the '895 patent. Claims 1-6 and 9-13 were rejected as anticipated by or obvious in light of many of the same prior art references relied on by the defendants in this case. After considering the patentee's arguments and declarations in support of patentability, the Patent Office issued a reexamination certificate and gave the following examiner's statement of reasons for patentability: "a method of preparing a food product wherein cruciferous sprouts, with the exception of cabbage, cress, mustard, and radish sprouts, that are rich in glucosinolates or contain high levels of phase 2 inducer activity are harvested prior to the 2-leaf stage is not taught or fairly suggested by the prior art or any combination thereof."

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Brassica also asks this court to construe the phrases "rich in glucosinolates" and "high Phase 2 enzyme-inducing potential" to require "at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential at 3-days following incubation under conditions in which cruciferous seeds germinate **[**12]** and grow." '895 patent, col. 7, ll. 47-53.

"The words of a claim are generally given their ordinary and accustomed meaning, unless it appears from the specification or the file history that they were used differently by the inventor." Carroll Touch, Inc. v. Electro Mech. Sys., Inc., 15 F.3d 1573, 1577, 27 USPQ2d 1836, 1840 (Fed. Cir. 1993). However, "limitations appearing in the specification will not be read into claims, and . . . interpreting what is *meant* by a word *in* a claim 'is not to be confused with adding an extraneous limitation appearing in the specification, which is improper.'" Intervet Am., Inc. v. Kee-Vet Labs., Inc., 887 F.2d 1050, 1053, 12 USPQ2d 1474, 1476 (Fed. Cir. 1989). Brassica's proposed construction violates this rule by improperly importing limitations from the specification into the claims. True, the specification states that "suitable sprouts will have at least 200,000 units per gram of fresh weight of Phase 2 enzyme-inducing potential following 3-days incubation of seeds under conditions in which the seeds germinate and grow." '895 patent, col. 10, l. 66 - col. 11, l. 2. The specification does not, however, indicate **[**13]** that the phrases "rich in glucosinolates" or "high in Phase 2 enzyme-inducing potential" are limited to these precise conditions. Rather, the specification uses the term "high" in its ordinary, comparative sense to mean "not low". For example, the specification states that "the cruciferous sprouts of the instant invention have higher Phase 2 enzyme-inducer potential than market stage plants," id. at col. 14, ll. 5-7, and the "Phase 2 enzyme-inducing potential of such sprouts may be as much as several hundred times higher than that observed in adult, market stage vegetables obtained from the same seeds," id. at col. 8, ll. 6-9; see also Innovad Inc. v. Microsoft Corp., 260 F.3d 1326, 1332, 59 USPQ2d 1676, 1680 (Fed. Cir. 2001) (construing the term "small volume" based in part on the specification's use of the phrase in its general sense to mean "not large"). Likewise, the term "rich" is not specifically defined or limited by the specification, but instead is used in its ordinary, relative sense. See, e.g., id. at col. 11, ll. 15-17 ("Mature Brussels sprouts and rapeseed are rich in these undesirable glucosinolates."); col. 11, ll. 37-39 ("Seeds, as well **[**14]** as sprouts have been found to be extremely rich in inducer potential.").

Brassica's proposed construction is also inconsistent with the language of the dependent claims. Claim 19 the '567 patent recites: "The method according to **[*1349]** claim 1, wherein said seeds produce cruciferous sprouts containing at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential measured after 3-days of growth." '567 patent, col. 22, ll. 62-65. Brassica's proposed construction would render this claim meaningless. See Comark Communications, Inc. v. Harris Corp., 156 F.3d 1182, 1187, 48 USPQ2d 1001, 1005-06 (Fed. Cir. 1998) (finding a violation of the doctrine of claim differentiation when a proposed construction would render another claim superfluous). We therefore reject Brassica's proposed claim construction for the phrases "rich in glucosinolates" and "high in Phase 2 enzyme-inducing potential."

II.

Having construed the claim limitations at issue, we now compare the claims to the prior art to determine if the prior art anticipates those claims. In order to prove that a claim is anticipated under 35 U.S.C. § 102 (b), defendants must present **[**15]** clear and convincing evidence that a single prior art reference discloses, either expressly or inherently, each limitation of the claim. Minn. Mining & Mfg. Co. v. Johnson & Johnson Orthopaedics, Inc., 976 F.2d 1559, 1565, 24 USPQ2d 1321, 1326 (Fed. Cir. 1992).

Brassica argues that the prior art does not expressly or inherently disclose the claim limitations of "preparing a food product rich in glucosinolates" (claims 1 and 9 of the '895 patent), or "identifying seeds which produce cruciferous sprouts . . . containing high Phase 2 enzyme-inducing potential" (claims 1 and 16 of the '505 patent, claim 1 of the '567 patent). According to Brassica, the prior art merely discusses growing and eating sprouts without mention of any glucosinolates or Phase 2 enzyme-inducing potential, and without specifying that particular sprouts having these beneficial characteristics should be assembled into a "food product." n3 Moreover, Brassica argues, the prior art does not inherently disclose these limitations because "at most, one following the prior art would have a possibility or probability of producing a food product high in Phase 2 enzyme-inducing potential" and the "fact that **[**16]** one following the prior art might have selected seeds meeting the limitations of the claims is not sufficient to establish inherent anticipation."

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n3 "A food product is any ingestible preparation containing the sprouts of the instant invention, or extracts or preparations made from these sprouts . . . ." '895 patent, col. 6, ll. 26-28.

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It is well settled that "a prior art reference may anticipate when the claim limitations not expressly found in that reference are nonetheless inherent in it. See, e.g., Atlas Powder Co. v. IRECO Inc., 190 F.3d 1342, 51 USPQ2d 1943 (Fed. Cir. 1999); Titanium Metals Corp. v. Banner, 778 F.2d 775, 227 USPQ 773 (Fed. Cir. 1985). "Under the principles of inherency, if the prior art necessarily functions in accordance with, or includes, the claimed limitations, it anticipates." MEHL/Biophile Int'l Corp. v. Milgraum, 192 F.3d 1362, 1365, 52 USPQ2d 1303, 1305 (Fed. Cir. 1999) (finding anticipation of a method of hair depilation by **[**17]** an article teaching a method of skin treatment but recognizing the disruption of hair follicles, citing In re King, 801 F.2d 1324, 1326, 231 USPQ 136, 138 (Fed. Cir. 1986)). "Inherency is not necessarily coterminous with the knowledge of those of ordinary skill in the art. Artisans of ordinary skill may not recognize the inherent characteristics or functioning of the prior art." MEHL/Biophile, 192 F.3d at 1365, 52 USPQ2d at 1305-06; **[*1350]** Atlas Powder, 190 F.3d at 1347, 51 USPQ2d at 1946-47.

Brassica does not claim to have invented a new kind of sprout, or a new way of growing or harvesting sprouts. Rather, Brassica recognized that some sprouts are rich in glucosinolates and high in Phase 2 enzyme-inducing activity while other sprouts are not. See '895 patent, col. 10, ll. 28-42 ("Sprouts suitable as sources of cancer chemoprotectants are generally cruciferous sprouts, with the exception of cabbage (Brassica olecracea capitata), cress (Lepidiumsativum), mustard (Sinapis alba and S. niger) and radish (Raphanus sativus) sprouts."). But the glucosinolate content and Phase 2 enzyme-inducing potential of sprouts necessarily have existed **[**18]** as long as sprouts themselves, which is certainly more than one year before the date of application at issue here. See, e.g., Karen Cross Whyte, The Complete Sprouting Cookbook 4 (1973) (noting that in "2939 B.C., the Emperor of China recorded the use of health giving sprouts"). Stated differently, a sprout's glucosinolate content and Phase 2 enzyme-inducing potential are inherent characteristics of the sprout. Cf. Brian R. Clement, Hippocrates Health Program 8 (1989) (referring to "inherent enzyme inhibitors, phytates (natural insecticides), oxalates, etc., present in every seed"). It matters not that those of ordinary skill heretofore may not have recognized these inherent characteristics of the sprouts. MEHL/Biophile, 192 F.3d at 1365, 52 USPQ2d at 1305.

Titanium Metals Corp. v. Banner is particularly instructive in this regard. In that case, the claim at issue recited:

> A titanium base alloy consisting essentially by weight of about 0.6% to 0.9% nickel, 0.2% to 0.4% molybdenum, up to 0.2% maximum iron, balance titanium, said alloy being characterized by good corrosion resistance in hot brine environments.

Titanium Metals, 778 F.2d at 776, 227 USPQ at 774. **[**19]** The prior art disclosed a titanium base alloy having the recited components of the claim, but the prior art did not disclose that such an alloy was "characterized by good corrosion resistance in hot brine environments." We nevertheless held that the claim was anticipated by the prior art, because "it is immaterial, on the issue of their novelty, what inherent properties the alloys have or whether these applicants discovered certain inherent properties." Id. at 782, 227 USPQ at 779. Titanium Metals explained the rationale behind this common sense conclusion:

> The basic provision of Title 35 applicable here is § 101, providing in relevant part: "Whoever invents or discovers any *new* . . . composition of matter, or any *new* . . . improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title."
>
> . . . .
>
> . . . Counsel never came to grips with the real issues: (1) what do the claims cover and (2) is what they cover new? Under the laws Congress wrote, they must be considered. Congress has not seen fit to permit the patenting of an old alloy, known to others through a printed publication, by one who has discovered **[**20]** its corrosion resistance or other useful properties, or has found out to what extent one can modify the composition of the alloy without losing such properties.

Id. at 780, 782, 227 USPQ at 777-78. Brassica has done nothing more than recognize properties inherent in certain prior art sprouts, just like the corrosion resistance properties inherent to the **[*1351]** prior art alloy in Titanium Metals. n4 While Brassica may have recognized something quite interesting about those sprouts, it simply has not invented anything new.

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n4 Most of the claims at issue are method claims, not composition or product claims. Nevertheless, the principles of Titanium Metals still apply. See, e.g., MEHL/Biophile, 192 F.3d at 1366-67, 52 USPQ2d at 1306 (finding anticipation by inherency of a method of hair depilation); Bristol-Myers, 246 F.3d at 1376, 58 USPQ2d at 1514 (Fed. Cir. 2001) (stating that "newly discovered results of known processes directed to the same purpose are not patentable because such results are inherent").

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Brassica nevertheless argues that its claims are not anticipated because the prior art does not disclose selecting the particular seeds that will germinate as sprouts rich in glucosinolates and high in Phase 2 enzyme-inducing potential (as opposed to selecting other kinds of seeds to sprout) in order to form a food product. We disagree. The prior art teaches sprouting and harvesting the very same seeds that the patents recognize as producing sprouts rich in glucosinolates and having high Phase 2 enzyme-inducing potential. According to the patents, examples of suitable sprouts are

> typically from the family Cruciferea, of the tribe Brassiceae, and of the subtribe Brassicinae. Preferably the sprouts are Brassica oleracea selected from the group of varieties consisting of acephala (kale, collards, wild cabbage, curly kale), medullosa (marrowstem kale), ramosa (thousand head kale), alboglabra (Chinese kale), botrytis (cauliflower, sprouting broccoli), costata (Portugese kale), gemmifera (Brussels sprouts), gongylodes (kohlrabi), italica (broccoli), palmifolia (Jersey kale), sabauda (savoy cabbage), sabellica (collards), and selensia (borecole), among others.

'895 patent, **[**22]** col. 10, ll. 32-42. Numerous prior art references identify these same sprouts as suitable for eating. See, e.g., Stephen Facciola, Cornucopia: A Source Book of Edible Plants 47 (1990) (listing "Brassica oleracea Botrytis Group - Cauliflower . . . Sprouted seeds are eaten"), Esther Munroe, Sprouts to Grow and Eat 9-14 (1974) (identifying "Broccoli, Brussels sprouts, Cabbage, Cauliflower, Collards and Kale"). These references therefore meet the claim limitation of identifying seeds to use in order to have sprouts with the inherent properties of glucosinolates and high Phase 2 enzyme-inducing activity. Despite the patents' admissions about the suitability of particular plant species found in these prior art references, Brassica argues that only specific cultivars of these plant species are rich in glucosinolates and high in Phase 2 enzyme-inducing activity. Thus, according to Brassica, the prior art fails to meet the "identifying" steps of the claims because it does not specify which cultivars should be sprouted. However, all of the appropriate cultivars that are identified in Brassica's patent are in the public domain. '895 patent, col. 10, ll. 43-65. Brassica cannot credibly **[**23]** maintain that no one has heretofore grown and eaten one of the many suitable cultivars identified by its patents. It is unnecessary for purposes of anticipation for the persons sprouting these particular cultivars to have realized that they were sprouting something rich in glucosinolates and high in Phase 2 enzyme-inducing potential. Atlas Powder, 190 F.3d at 1348, 51 USPQ2d at 1947 ("The public remains free to make, use, or sell prior art compositions or processes, regardless of whether or not they understand their complete makeup of the underlying scientific principles which allow them to operate.").

The prior art also discloses the remaining limitations of the claims. The Munroe **[*1352]** reference, for example, recommends that sprouts be harvested between "3 to 5 days for a sprouted length of 1/2 to 1 inch." Munroe at 9. Photographs of these sprouts show that they have not yet reached the two-leaf stage of development. Id. at 10-13. Thus, this reference discloses the claim limitations of germinating the appropriate cruciferous seeds and harvesting the resulting sprouts prior to the 2-leaf stage. See '895 patent, claims 1 and 9; '567 patent, claims 1 and 2; '505 **[**24]** patent, claims 1 and 16. Munroe also discloses that these particular sprouts can be used in food products such as "soups, salads and main dishes," id. at p. 14, thereby meeting the claim limitation of forming a food product comprising a plurality of the sprouts ('895 patent claims 1 and 9; '567 patent, claims 1 and 8; '505 patent, claims 1 and 16) and the claim limitation of administering (eating) the food product ('505 patent, claims 1 and 16). The Munroe reference therefore discloses each and every limitation of these claims of the patents. See also, Meyerowitz, Growing Vegetables Indoors (1990).

In summary, the prior art inherently contains the claim limitations that Brassica relies upon to distinguish its claims from the prior art. While Brassica may have recognized something about sprouts that was not known before, Brassica's claims do not describe a new method.

CONCLUSION

For the foregoing reasons, we affirm the district court's summary judgment that the claims at issue are anticipated by the prior art. The prior art indisputably includes growing, harvesting and eating particular sprouts which Brassica has recognized as being rich in glucosinolates and high in **[**25]** Phase 2 enzyme-inducing potential. But the glucosinolate content and Phase 2 enzyme-inducing potential of these sprouts are inherent properties of the sprouts put there by nature, not by Brassica. Brassica simply has not claimed anything that is new and its claims are therefore invalid.

AFFIRMED